VOLUME LXXII NUMBER 5

Hospital Library

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

May, 1943

Effect of Desoxycorticosterone Acetate Administration on Plasma Volume and Electrolyte Balance of Normal Human Subjects. MARSHALL CLINTON, JR., AND GEORGE W. THORN 255

Behavior of Bipedal Rats. LORING W. PRATT 265

Studies in Experimental Hypertension. II. The Effect of Dietary Protein on the Urea Clearance and Arterial Blood Pressure in Chronic Hypertension. LOUIS K. ALPERT AND CAROLINE B. THOMAS 274

Studies in Experimental Hypertension. III. The Effect of Dietary Protein on the Clearances of Diodrast and Inulin by the Kidney in Chronic Hypertension. LOUIS K. ALPERT AND JOSEPH L. LILIENTHAL, JR. 286

Endocardial Fibroelastosis (So-Called Fetal Endocarditis). A Report of Two Cases Occurring in Siblings. TOBIAS WEINBERG AND ALBERT J. HIMELFARB 299

Books Received for Review 307

PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore, Maryland

Made in United States of America

COPYRIGHT, 1943, BY THE JOHNS HOPKINS PRESS

BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

MANAGING EDITOR, MAXWELL M. WINTROBE

EDITORIAL BOARD

E. Cowles Andrus
Charles R. Austrian
W. Halsey Barker
James Bordley, III
Chalmers L. Gemmill
L. Emmett Holt, Jr.
Arnold R. Rich

All correspondence relating to the publication of papers should be addressed to The Editor, Bulletin of The Johns Hopkins Hospital, Baltimore, Md.

Books for review should be sent to The Editor at the above address.

Twenty-five reprints, without covers, of articles will be furnished to contributors free of cost. An order slip for additional reprints, with a table showing cost, is sent with proof.

Correspondence concerning business matters should be sent to The Johns Hopkins Press, Baltimore, Maryland, U. S. A.

This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro rata value of such numbers will be credited to the next volume, and the renewal invoice therefor adjusted accordingly.

Subscriptions should be renewed promptly. To avoid a break in your series, subscriptions should be renewed promptly. The publisher cannot guarantee to supply back issues on belated renewals.

Subscriptions, new or renewal, may be sent to the Publishers or ordered through your agent.

Subscriptions in the British Empire except Canada may be sent to: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, London, W.C. 2.

THE JOHNS HOPKINS PRESS
BALTIMORE, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Postoffice. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

EFFECT OF DESOXYCORTICOSTERONE ACETATE ADMINISTRATION ON PLASMA VOLUME AND ELECTROLYTE BALANCE OF NORMAL HUMAN SUBJECTS[1, 2]

MARSHALL CLINTON, JR.[3], AND GEORGE W. THORN[4]

From the Chemical Division, Medical Clinic, The Johns Hopkins University and Hospital, Baltimore, Maryland

Received for publication March 24, 1943

This study was undertaken to determine whether or not the administration of desoxycorticosterone acetate increased plasma volume in normal subjects. It is well known that synthetic adrenal hormone therapy induces a prompt and striking rise in plasma volume in patients with Addison's disease (1, 2). Few observations on the effect of desoxycorticosterone acetate therapy in normal subjects have been made (3). In the present study the effect of intramuscular injection of synthetic hormone in oil has been compared with sublingual administration of tablets of crystalline hormone.[5] Both methods of hormone administration have been studied with and without supplementary sodium chloride administration. A search has been made for the possible toxic effects of desoxycorticosterone acetate treatment.

METHODS

The subjects were normal, healthy male students who were given a careful physical examination at the beginning of this study. The medical examination included a teleoroentgenogram of the chest, blood count, blood chemical studies and urinalysis. Throughout the experimental period each subject was maintained

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Johns Hopkins University.

[2] This study was aided in part by a grant from the Committee on Research in Endocrinology, National Research Council.

[3] John D. Archbold Fellow in Medicine.

[4] Present address: Department of Medicine, Harvard Medical School and the Peter Bent Brigham Hospital, Boston, Massachusetts.

[5] "Linguets"—tablets composed of crystalline desoxycorticosterone acetate, lactose, sucrose, talcum and gum arabic for sublingual use were kindly supplied by Dr. E. Oppenheimer of the Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

on a diet of constant composition. The diet contained 5.0 gm. of carbohydrate and 1.3 gm. of protein per kilogram of body weight. The amount of fat in the diet, usually 1.5 gm. per kg. body weight, was adjusted to meet caloric requirements. Fluid intake was maintained at a constant level for each subject.

Twenty-four hour urine specimens were collected under toluol and analyzed for sodium (4) and chloride (5). The accuracy of these collections was checked by determining the creatinine content (6) of the urine specimens. Specimens of blood for chemical analyses, hematocrit and red blood cell determinations were withdrawn from the antecubital vein *under basal conditions following a 12-hour fast* at the beginning and termination of each treatment period. Plasma volume determinations were made according to the method of Gregersen and Gibson (7, 8), using the blue dye, T-1824. The concentration of dye in serum was determined by means of the Evelyn photoelectric colorimeter (9, 10). Changes in plasma volume during short periods of treatment (3–5 days) were calculated from changes in hematocrit. As a check on the validity of this calculation it was shown that values determined from the hematocrit and a previous plasma volume measurement (T-1824) differed by less than 3.5 per cent from those obtained by a recent direct determination of plasma volume when both determinations were made under *carefully controlled basal conditions.*

OBSERVATIONS

1. The Effect of Single Daily Intramuscular Injections of Synthetic Desoxycorticosterone Acetate in Oil (Percorten—Ciba)

A striking increase in plasma volume was noted in all three of the normal subjects treated with 10 mg. of desoxycorticosterone acetate in oil once daily (table 1). In all instances the maximum increase in plasma volume was observed on the fourth day of therapy. The greatest increase noted was 740 cc., an increase equal to 26 per cent of the original plasma volume of this subject (W. S.). The average increase in the plasma volume in the three subjects was 560 cc. or 17.5 per cent of the original plasma volume. Following the withdrawal of therapy a decrease in plasma volume was noted within 24 hours. The plasma volumes in all three subjects had returned to normal values or lower by the third day after treatment had been discontinued.

A marked retention of sodium and chloride occurred during the five-day period of treatment. The average total retention amounted to 170 m.eq. of sodium and 92 m.eq. of chloride during this period. The reduced output of sodium and chloride persisted throughout the entire five days of therapy. A gain in body weight of 0.4 kg. (average)

TABLE 1

Plasma volume changes associated with the daily intramuscular administration of 10 mg. of synthetic desoxycorticosterone acetate in sesame oil (percorten—Ciba)

(Normal male subjects)

SUBJECT	PLASMA VOLUME (CC.)					MAXIMUM INCREASE NOTED
	Prior to therapy	2nd day of therapy	3rd day of therapy	4th day of therapy	5th day of therapy	
						cc.
J. B.	3625	3655	3900	4250	4230	625
T. H.	3190	3260	3430	3490	3450	300
W. S.	2770		3270	3520	3460	740

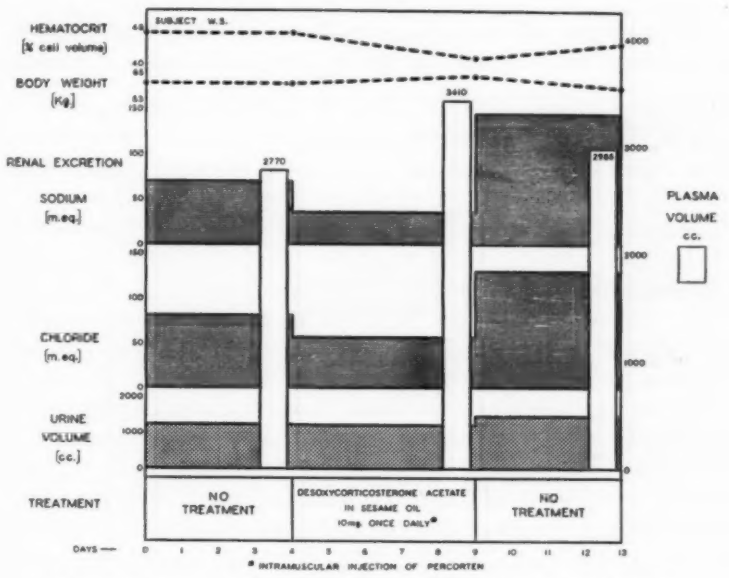

CHART 1. EFFECT OF INTRAMUSCULAR ADMINISTRATION OF DESOXYCORTICOSTERONE ACETATE ON PLASMA VOLUME AND ELECTROLYTE BALANCE

accompanied the retention of sodium, chloride and water. No significant change in blood pressure was observed in any of the subjects. Withdrawal of therapy was followed by an increased excretion of

sodium, chloride and water, and a decrease in body weight. The increased excretion of sodium, chloride and water became apparent on the first day following withdrawal of treatment and reached its maximum on the second day. By the fifth day following withdrawal of treatment, the urinary sodium and chloride values had returned to the pre-treatment levels. The changes in plasma volume and electrolyte balance noted in subject (W. S.) treated with 10 mg. of desoxycorticosterone acetate daily for a five-day period are shown in Chart 1.

2. The Effect of Hormone Administered Sublingually in Divided Doses ("Linguets"—Ciba)

Three normal subjects were given 30 mg. of hormone sublingually in divided doses (one 10 mg. tablet three times daily). A fourth

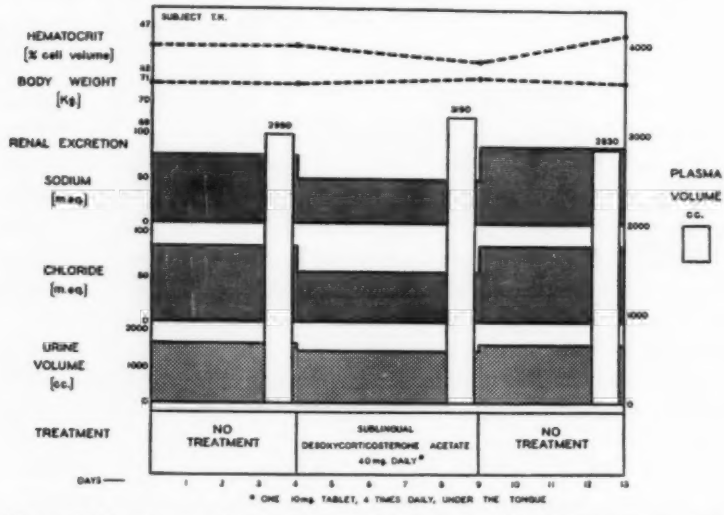

CHART 2. EFFECT OF SUBLINGUALLY ADMINISTERED DESOXYCORTICOSTERONE ACETATE ON PLASMA VOLUME AND ELECTROLYTE BALANCE

subject received 10 mg. four times daily (40 mg. total daily dose). The average increase in plasma volume which occurred in these four subjects during sublingual therapy was 200 cc. (approximately 7 per cent of the initial plasma volume). The maximum increase in plasma

volume observed during *sublingual* therapy (40 mg. daily) was 215 cc. This was considerably less than the increase noted during the daily intramuscular administration of 10 mg. of hormone in oil. The time relationship in plasma volume changes associated with sublingual administration of hormone paralleled those observed during intramuscular administration of hormone. The effect of sublingually administered hormone on electrolyte balance, plasma volume, hematocrit values and body weight is illustrated in Chart 2. These changes paralleled those observed during intramuscular therapy but were much less striking.

3. A Comparison Between the Effect of Synthetic Adrenal Cortical Hormone Therapy with and without Supplementary Sodium Chloride Medication

In these experiments, each subject was given 10 mg. of desoxycorticosterone acetate in sesame oil intramuscularly each morning, in addition to 6 gm. of supplementary sodium chloride orally in divided doses during the day, for a five-day period. An average increase in plasma volume of 540 cc. occurred in these experiments (table 2). This represented an increase of 16 per cent over the original plasma volume. This value corresponds closely to that observed in the three subjects who were given intramuscular injections of hormone in oil without supplementary sodium chloride. It is evident that although substantial increases in plasma volume had occurred after two days of therapy, further increases followed continuation of therapy (Chart 3). In those subjects in whom plasma volume determinations were made on the fourth day of treatment, the value was greater than that observed on the fifth day of therapy. No significant changes in blood chemistry were noted although three subjects showed a slight rise in serum sodium and chloride levels. Changes in red blood cell counts and hemoglobin determinations paralleled the changes in hematocrit values, but no changes in mean corpuscular volume or mean corpuscular hemoglobin were observed. Marked retention of sodium and chloride occurred during the period of hormone administration. Approximately 15 gm. (50 per cent) of the supplementary sodium chloride was retained by all four of the subjects. An increase in body weight (0.9 kg. average) accompanied the retention of sodium and chloride. The excess sodium and chloride retained during the treatment period

was excreted during the first four days following the withdrawal of therapy. It is evident that the administration of supplementary

TABLE 2

Plasma volume changes associated with the daily intramuscular administration of 10 mg. of desoxycorticosterone acetate in sesame oil and 6 grams of supplementary sodium chloride by mouth

(Normal male subjects)

SUBJECT	PLASMA VOLUME (CC.)					MAXIMUM INCREASE NOTED
	Prior to therapy	2nd day of therapy	3rd day of therapy	4th day of therapy	5th day of therapy	
						cc.
A. G. H.	4030		4445		4790	760
W. S.	2845	3215		3230	3135	295
J. B.	3130	3665	3855		3705	725
T. H.	3170	3435		3540	3435	370

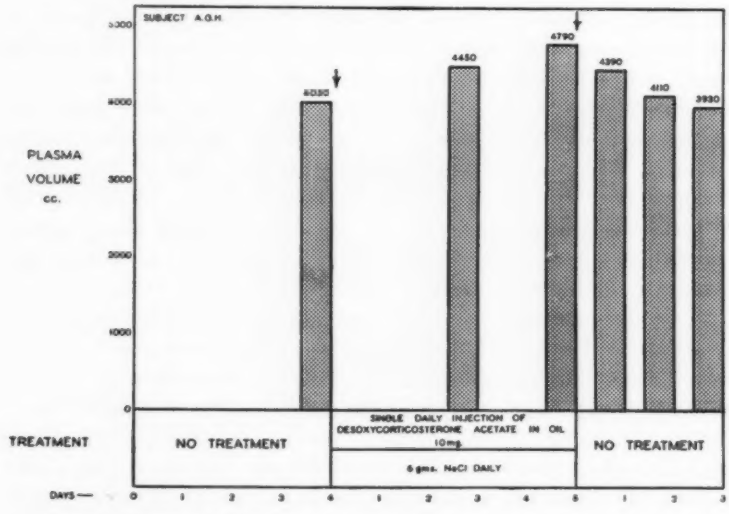

CHART 3. EFFECT OF INJECTED DESOXYCORTICOSTERONE ACETATE AND SUPPLEMENTARY SODIUM CHLORIDE ON PLASMA VOLUME

sodium chloride did not augment the plasma volume increase noted following the administration of single daily injections of 10 mg. of

desoxycorticosterone acetate. Sublingual administration of 30 mg. of desoxycorticosterone acetate (10 mg. three times daily) in addition to 6 gm. of supplementary sodium chloride medication was followed by an average increase of 50 cc. in plasma volume (2 per cent of original plasma volume). This change is well within the range of experimental error.

4. The Effect of Sodium Chloride Therapy Alone on Plasma Volume

The administration of 6 gm. of supplementary sodium chloride daily for a period of five days was followed by an average increase in plasma volume of 190 cc. (6 per cent of the original plasma volume) in three subjects.

5. A Search for Evidence of Toxicity

None of the subjects showed any evidence of peripheral edema, dyspnea, gastro-intestinal disturbances or other untoward reactions during the five-day period of desoxycorticosterone acetate administration (10 mg. once daily intramuscularly; 30–40 mg. daily sublingually).

In order to determine whether or not more prolonged administration of desoxycorticosterone acetate in relatively large doses had any deleterious effects, one subject (J. B.) was given a daily intramuscular injection of 10 mg. of hormone for a period of fifteen days. In addition, he received 6 gm. of supplementary sodium chloride daily. During this period of prolonged therapy no unpleasant symptoms occurred. No significant change in blood pressure level was noted and there was no edema. Electrocardiograms taken prior to and at the end of treatment showed no difference, and the heart size as measured by x-ray was not significantly greater. There was a rapid increase in plasma volume during the first four days of treatment, followed by a much smaller but continued increase during the remainder of the period. One interesting observation in this subject was the increase in volume of red blood cells from 2,965 cc. at the start of therapy to 3,200 cc. at the end of 15 days of treatment. The increase in red blood cell volume appeared to be dependent in large measure on an increase in the number of red blood cells rather than an increase in mean corpuscular volume.

DISCUSSION

It was apparent from these studies that the daily intramuscular injection of 10 mg. of desoxycorticosterone acetate in oil was followed by a significant increase in plasma volume in normal male subjects. The maximum increase in plasma volume occurred on the fourth day of treatment. Continued treatment did not result in a further increase in plasma volume. Supplementary sodium chloride medication did not augment the increase in plasma volume which occurred during synthetic hormone administration, but did increase appreciably the total quantity of sodium, chloride and water which was retained. The relative inefficacy of administering desoxycorticosterone acetate sublingually is clearly demonstrated (11).

Although no evidences of toxic symptoms were encountered with the moderate dosage employed over this relatively short period of treatment, it should be emphasized that severe toxic effects have been observed in normal animals treated with large doses of hormone for prolonged periods (12, 13). Similar reactions have been encountered in patients with Addison's disease who have received excessive quantities of desoxycorticosterone acetate and supplementary sodium chloride (2, 14, 15).

SUMMARY AND CONCLUSIONS

1. The daily intramuscular administration of 10 mg. of desoxycorticosterone acetate in sesame oil to four normal subjects resulted in a considerable increase in plasma volume in all. The maximum increase occurred on the fourth day of treatment. The increase persisted throughout the period of therapy (five–fifteen days). Following withdrawal of treatment, plasma volume fell to normal or slightly below within a period of three days. The magnitude of the changes in plasma volume was not significantly influenced by diet or supplementary sodium chloride administration.

2. The sublingual administration of 30–40 mg. of desoxycorticosterone acetate in divided doses was much less effective than 10 mg. once daily intramuscularly in inducing an increase in plasma volume in normal subjects.

3. Although the simultaneous administration of supplementary sodium chloride did not augment the increase in plasma volume fol-

lowing desoxycorticosterone acetate therapy, the total quantity of sodium, chloride and water which was retained during the period of synthetic hormone treatment was increased appreciably.

4. Supplementary sodium chloride alone was followed by a slight increase in plasma volume.

5. No evidences of toxic symptoms were observed in normal subjects treated with desoxycorticosterone acetate during a relatively short period (five to fifteen days).

Acknowledgments

The authors are indebted to Mrs. Kay E. Stein, Chemical Technician; Miss Rachel Fee, Nurse-in-charge, and Miss Janet Engebretsen, Dietitian-in-charge of the Metabolism unit; also to Messrs. J. R. Birmingham, T. L. Hartman, G. J. Hayes, A. G. Hills, B. Katzin, T. J. Kennedy, D. B. Miller and W. H. Sause, who kindly volunteered as subjects for this study.

BIBLIOGRAPHY

1. Thorn, G. W., Howard, R. P., and Emerson, K., Jr.: Treatment of Addison's Disease with Desoxycorticosterone Acetate, A Synthetic Adrenal Cortical Hormone (Preliminary Report). *J. Clin. Investigation*, **18:** 449, 1939.
2. Ferrebee, J. W., Ragan, C., Atchley, D. W., and Loeb, R. F.: Desoxycorticosterone Esters: Certain Effects in the Treatment of Addison's Disease. *J. A. M. A.*, **113:** 1725, 1939.
3. Thorn, G. W., and Emerson, K., Jr.: The Role of Gonadal and Adrenal Cortical Hormones in Production of Edema. *Ann. Int. Med.*, **14:** 757, 1940.
4. Butler, A. M., and Tuthill, T.: An Application of the Uranyl Zinc Acetate Method for Determination of Sodium in Biological Material. *J. Biol. Chem.*, **93:** 171, 1931.
5. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry*. Vol. II. Methods. Chapter 30, Chloride. Williams & Wilkins Co., Baltimore, 1932.
6. Folin, O.: On the Determination of Creatinine and Creatine in Urine. *J. Biol. Chem.*, **17:** 469, 1914.
7. Gregersen, M. I., and Gibson, J. G., 2d: Conditions Affecting the Absorbtion Spectra of Vital Dyes in Plasma. *Am. J. Physiol.*, **120:** 494, 1937.
8. Gibson, J. G., 2d, and Evans, W. A., Jr.: Clinical Studies of the Blood Volume. I. Clinical Application of a Method Employing the Azo Dye "Evans Blue" and the Spectrophotometer. *J. Clin. Investigation*, **16:** 301, 1937.
9. Gibson, J. G., 2d, and Evelyn, K. A.: Clinical Studies of the Blood Volume. IV. Adaptation of the Method to the Photoelectric Microcolorimeter. *J. Clin. Investigation*, **17:** 153, 1938.

10. EVELYN, K. A., AND CIPRIANI, A. J.: A Photoelectric Microcolorimeter. *J. Biol. Chem.*, **117:** 365, 1937.
11. THORN, G. W., DORRANCE, S. S., AND DAY, E.: Addison's Disease: Evaluation of Synthetic Desoxycorticosterone Acetate Therapy in 158 Patients. *Ann. Int. Med.*, **16:** 1053, 1942.
12. KUHLMAN, D., RAGAN, C., FERREBEE, J. W., ATCHLEY, D. W., AND LOEB, R. F.: Toxic Effects of Desoxycorticosterone Esters in Dogs. *Science*, **90:** 496, 1939.
13. DARROW, D. C., AND MILLER, H. C.: The Production of Cardiac Lesions by Repeated Injections of Desoxycorticosterone Acetate. *J. Clin. Investigation*, **21:** 601, 1942.
14. THORN, G. W., AND FIROR, W. M.: Desoxycorticosterone Acetate Therapy in Addison's Disease. Clinical Considerations. *J. A. M. A.*, **114:** 2517, 1940.
15. WOOD, W. B., JR., AND MOORE, R. A.: Case Reports of the Barnes Hospital. Case 10. *J. Missouri M. A.*, page 48, February 1943.

BEHAVIOR OF BIPEDAL RATS

LORING W. PRATT[1]

Department of Anatomy, Johns Hopkins University

Received for publication February 12, 1943

The fore limbs of puppies were removed by Knickmeyer (in Fuld, 1901) and by Fuld (1901). These investigators studied the resultant change in the tibio-femoral index. A dissection of the muscular and osseous anatomy of a dog born without fore limbs was made by Regnault in 1911. Colton (1929) decided to study bipedalism in an animal that allowed of statistical consideration, and picked the rat. He was concerned primarily with the effects of bipedal habit upon the bones of the hind legs.

In his original study, Colton did not remove much of the brachium; he ligated the axillary artery and transected the humerus just above the elbow. From his studies, and those of Knickmeyer, Fuld and Regnault, it was determined that the tibio-femoral index of the biped dog is altered in the direction of the kangaroo, while that of the biped rat is changed in the direction of man. They further attempted correlation of these facts with the statements that dog and kangaroo are both digitigrade, while man and rat are plantigrade animals. It is evident that this is not entirely correct as the kangaroo is a plantigrade animal, like man and rat, becoming digitigrade only when travelling rapidly.

Henry Fairfield Osborn (1922) pointed to the theoretical importance of these studies as a possible means of shedding light upon some of the developmental trends of animals throughout the history of vertebrate evolution.

In the above studies, little attention was paid to the activity of the animals under consideration. Colton reported briefly that the bipedal rats walk like man, taking relatively shorter steps than the quadrupeds, and also that they sometimes push themselves along the floor of the cage by means of their hind feet. He reported one female rat that repeatedly jumped ten inches to the rim of a bucket,

[1] Henry Strong Denison scholar for 1942–43.

where she balanced satisfactorily. In ordinary life they appeared to be fully as active as the controls.

This work was begun to ascertain facts of interest about behavior not covered by previous workers with the bipedal mammals. It was thought that it would be interesting to determine the effect upon the activity of the rat and upon its anatomy of more complete extirpation of the pectoral girdle. Observations were made especially with regard to the locomotion of the experimental animals; the face-washing reflex, their ability to handle food, to reproduce and then care for young.

MATERIAL AND METHODS

Week old laboratory rats were selected and while under ether anesthesia the axillary artery was ligated and the fore limb excised. The amount of appendage removed varied with the individual. In some instances the shoulder was disarticulated and the brachium and antebrachium were amputated. In others the scapula was added to this list, while in still others the clavicle also was eliminated. In several specimens, brachium, antebrachium, scapula, and clavicle were removed, with their attached musculature. These operations were done unilaterally on the entire litter at the age of one week. At two weeks, the rats were again subjected to operation and the remaining fore limbs extirpated. By doing this work in two stages, mortality was negligible. In no case was postoperative infection noted.

Recovery following operation was uneventful, very rapid, and in many instances it was observed that within fifteen minutes of the second operation the individual sat up and assumed a posture similar to that of the adult biped. When the entire litter was operated on, little or no trouble was encountered, but in mixing them with normal young much difficulty was experienced, as these rats lacked fore limbs with which to compete for the nipple. Following the plan of operating upon entire litters at one time the young were fat and well nourished. Special care was given to see that they remained that way, by supplying nourishing foods and the advantages of larger, less crowded cages.

POSTURE

The posture of these animals is characteristic. In ordinary, quiet life, it is altered from that of the normal rat in that the weight is thrown farther back on the haunches and the animal develops an exaggerated kyphosis, especially of the lower thoracic and upper lumbar regions. This lends a penguin- or kiwi-like appearance to the resting position. If they have the proper stimulus, they are perfectly able to stand straight with apparent ease, and even to

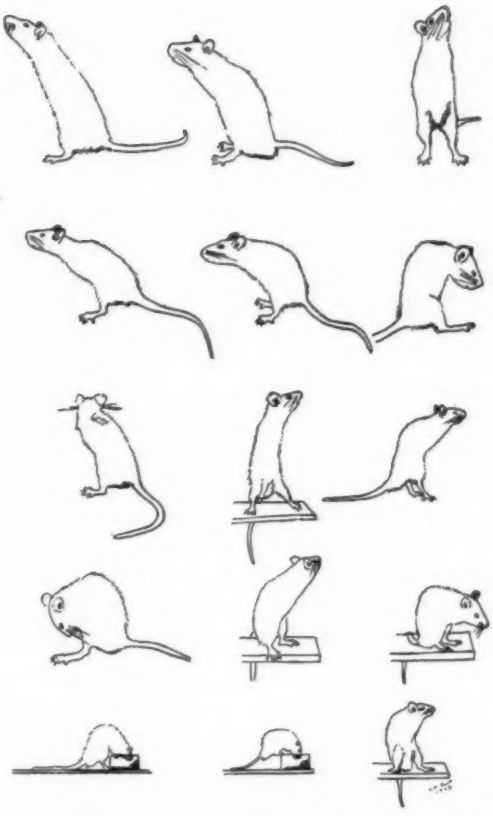

FIG. 1. COMMON VARIATIONS IN THE POSTURES ASSUMED BY BIPED RATS

It should be noted that at times the foot is used in plantigrade manner, but at other times it is functionally digitigrade. The drawings were made from photographs.

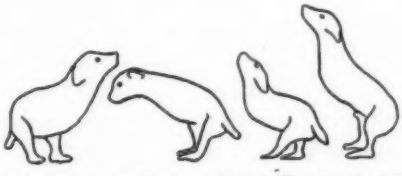

FIG. 2. POSTURES ASSUMED BY BIPEDAL DOGS, TRACED FROM PHOTOGRAPHS USED BY FULD, 1901

become temporarily digitigrade, standing on tiptoe and balancing on toes and tail, tripod-fashion. When standing in this way, they occasionally lean so far backwards that they look as though they must surely fall over, but no difficulty of that sort was ever observed. When sleeping, they not uncommonly lie on their backs with both hind feet in the air.

Part of the change in posture is attributable to the shift in the center of gravity of the biped rats, consequent to their support by the hind limbs only, and a part to the fact that when the animals are engaged in feeding or other activities involving the head, there are no fore limbs to help. This shift in the center of gravity is well shown by the illustration of rats and of Fuld's dogs (figures 1 and 2). In the rat, the hips are relatively heavier than in the dog, and in addition to this the rat has a long tail which is used effectively as a counterweight. This enables the rat to eat, and to touch the ground with the nose by flexion of the vertebral column. For Fuld's dogs to do this it would have been necessary for them to flex the thighs sharply at the hips and then lean forward. The fact that the rat develops extreme kyphosis is due in large measure to the fact that its center of gravity is normally located nearer the hips, perhaps chiefly because of the heavier tail, which shifts the center of gravity to the rear.

LOCOMOTION

Bipedal rats adjust themselves to a new method of locomotion very rapidly. The little rats, after recovering from the second operation (in 24 hours or so), hop around on their hind feet kangaroo-like or else walk as a man, with great facility. By using their tails to lean on, tripod fashion, they can stand almost vertically. As they grow older, they develop three definite methods of locomotion. They may walk like a man when they are unhurried. Sometimes they lean forward and "snowplow" along the floor of the cage, utilizing any of the pectoral girdle that may not have been excised. This is a snake-like mode of travel and is especially common in those animals still having scapulae and clavicles. They make considerable use of these bones in slow travel, walking on the shoulder stumps. When they are in a hurry, and desire to move rapidly, they hop with a kangaroo-like gait, by which process they make very rapid progress.

They learn to jump vertically very easily, and without difficulty can be taught to jump 12 or 14 inches to a shelf upon which the food dish has been placed, or even to a series of such shelves, staggered one above the other. Ordinary rats would not jump to such a height, but would climb the side of the cage to obtain the food.

In descending from a shelf, two methods are used by bipedal rats. Much caution is employed in jumping down, as they find that to be hard upon the end of the nose or chest due to a lack of front feet to break the fall. As a result they either jump straight away from the shelf and "pancake" to the floor, apparently endeavoring to get their hind feet in under them; or they hang by the hind feet, dangle down as far as possible, and then drop upon the chest. This latter is probably the most common method of leaving a shelf. The jumping is accurate, and in leaping to a narrow ledge eight inches from the floor, the rat lands without difficulty, and apparently has no trouble in maintaining its balance upon landing.

It would be interesting to record observations comparing the relative locomotor ability of bipedal and quadrupedal rats. I have obtained no quantitative measurements of relative speeds; but I made one observation of interest. Four rats escaped from the colony and took up residence under a chest that was blocked up off the floor. After unsuccessfully trying to catch them for four days, in which time they all became quite wild, the chest was surrounded by a board wall and then lifted out, leaving a pen about four feet square in which the rats could run. The quadrupedal animal was caught with ease. A claviclectomized animal was captured with some trouble, and two bipeds were captured only with considerable effort. The impression was that the latter could travel better because they no longer had front feet to interfere with their progress. While this is not quantitative, it is interesting to note that the kangaroo type of locomotion is adapted to high speed travel by the biped rat. This is especially interesting in view of Colton's findings that the tibio-femoral index of the rat is changed toward that of man and away from that of the kangaroo by adaptation to the biped habit. Although man-like locomotion is commonly used by the two-legged rat, when high speed is required, he utilizes the kangaroo's method of travel. This is in spite of the rat's essentially digitigrade gait, when hopping rapidly.

FACE-WASHING REFLEX AND PHANTOM LIMBS

Perhaps the most interesting alteration in function is that of the "washing reflex" in the rat. This washing of the face, nose and head has been observed by the author to be present in young normal individuals as early as the fifth day. This consists (in the adult) of sitting on the hind legs, rubbing the teeth, lips, and nose with the forefeet with occasional movements in which the rat passes the hands over head, eyes and ears, and back again over the face. This ostensibly is an act of grooming, and was found to be present in its essentials in the rat with no fore limbs. It was first noticed with reference to the special diet fed to one rat.

Due to the fact that particular care was taken with rat number 43 it was thought well to feed it raw Norwegian cod liver oil. This was administered every other day by means of a medicine dropper, and, in order to reduce the possibility of producing lipoid pneumonia, the oil was smeared on the lips or tongue of the rat, never being injected into the mouth.

Following the administration of the oil, the rat regularly sat upon its hind legs, twisted its head from side to side, moved its scapulae and clavicles and used them synchronously as though it were washing its face. The scapulae moved in a rotary motion, as a normal rat moves them when washing, nose held up, neck arched and chin in. The head gyrated as though the hands were running over the nose, eyes, ears and grooming the face. In all ways, the biped resembled the normal animal in washing its face, except that it had no fore limbs to manipulate.

After making this observation, it was frequently observed that in eating and in other normal activities the scapulae were used much as though they were connected with arms. The most striking and extreme illustration of this is seen in animals from which the scapulae, clavicles and arms have been removed. In these the movements of the subcutaneous tissues, as indicated by skin motion when the animal is "washing his face" or in the process of eating, are precisely the same as those seen in animals which have part of the pectoral girdle intact. This shows clearly and graphically that it is not necessary for the muscles to be attached to the bones of the pectoral girdle to allow for this type of motion.

The reflexes here operating in the case of bipedal rats must be akin to "phantom limb" reflexes commonly encountered in humans after amputation of a limb. This phenomenon has been considered by Riddoch (1941) who concludes that there are two factors concerned with the phantom extremity. In the first place, from the time of birth or before, the individual receives sensory stimuli from all parts of the body at all times, and especially from the ends of the extremities. These are fused to form what Head and Holmes (1911–1912) call a "plastic model" or "schema" of the surface of the body. These models are built up chiefly through perception of skin areas, and through postural relationships. We have demonstrated that rats that have had their fore limbs amputated in infancy, before the development of a plastic model is likely, apparently have the ability to use their phantom limbs in grooming. It would seem, then, that stimulation of the upper lip of the rat sets off stimuli that, by reflex action, cause the rat to wash its face with the phantom limbs. Although the limbs are gone, there probably remains an awareness of them. In the human phantom limb, the patient realizes that an attempt to use his foot, for instance, is foolish, and therefore he becomes conditioned to ignoring the reaction until he suppresses the desire to utilize the phantom. An animal such as the rat is relatively less inhibited about such matters, and therefore the functional phantom may be much more persistent and obvious than in the human.

REPRODUCTION

No difficulties were observed in reproducion in biped rats. By virtue of persistence and resourcefulness the male was able successfully to manage coitus in spite of his lack of fore limbs. The female was usually crowded in a corner, and then held by the male placing head and neck on her shoulders in such a way as to keep her from escaping. This is similar to the behavior of snakes. In two instances bipedal males impregnated normal females.

The females carried and delivered young without incident in most cases. Two parturitions were fatal to the female, but in both instances it was thought that this was caused by injury to the unborn young, or possibly to the female, by the unusual strain upon these animals. These females were both very lively and given to hopping actively about the cage. In five or six times as many deliveries among

quadrupedal females during the same period of time, only two females died in parturition.

The female had no difficulty in raising the young after they were born. They suckled normally; in no case were any of the axillary nipples damaged at operation. The young grew at the same rate as those from normal mothers, and gave no evidence of any nutritional difficulty. They, of course, were all normal quadrupedal infant rats.

Anatomical studies have been begun on the material obtained from these animals but are, as yet, incomplete.

CONCLUSIONS

Biped habit is rapidly and easily acquired by young rats subjected to operative removal of the pectoral girdle. These rats continue to go through all the central maneuvers of face washing, apparently with the aid of phantom limbs, although without any peripheral limbs to pass over the face.

SUMMARY

Normal laboratory rats were operated upon at a week of age and the pectoral girdle removed in two stage operations. These rats grew normally and showed no essential lack in their activities. The normal posture is with the back arched and the weight thrown back on the haunches. Special modes of locomotion, man-like, kangaroo-like, and snake-like were utilized by the rats. They were easily trained to jump to considerable heights in search of food. Descent from a shelf was the most difficult activity for them. Persistence of the "face washing reflex" was observed in spite of complete absence of forelimbs and pectoral girdle, the activities being strongly suggestive of the presence of phantom limbs. Reproduction appeared to be normal in these animals.

The author gratefully acknowledges the guidance and help given him in this work by A. Brazier Howell.

BIBLIOGRAPHY

COLTON, H. S.: How bipedal habit affects the bones of the hind legs of the albino rat. *Jour. Exper. Zool.*, **53**, 1, 1929.

————: Biped habit. *Sci. Monthly*, **31**, 81, 1930.

FULD, E.: Über Veränderungen der Hinterbeinknochen von Hunden in Folge mangels der Vorderbeirne. Beitrage zur Frage nach den Ursachen der Knochengestaltung und zur Transformationslehre der Organismen. *Arch. Entw. Org.*, **2**, No. 1, S. 61, 1901.

HEAD, H. AND G. HOLMES: Sensory disturbances from cerebral lesions. *Brain*, **34**, 102, 1911–12.

OSBORN, H. F.: Orthogenesis as observed from paleontological evidence beginning in the year 1889. *Amer. Nat.*, **56**, 134, 1922.

REGNAULT, F.: The ectromelous dog and the theories of Lamarck. *Biologica*, **1**, 333, 1911.

RIDDOCH, G.: Phantom limbs and body shape. *Brain*, **64**, 14, 1941.

STUDIES IN EXPERIMENTAL HYPERTENSION[1]

II. The Effect of Dietary Protein on the Urea Clearance and Arterial Blood Pressure in Chronic Hypertension[2]

LOUIS K. ALPERT, M. D., AND CAROLINE B. THOMAS, M.D.

From the Department of Medicine, the Johns Hopkins University School of Medicine

Received for publication January 26, 1943

The observations described in the preceding paper of this series[3] indicated that in dogs with chronic hypertension the renal blood flow, under basal conditions, was normal. However, the possibility was not excluded that the vascular supply to the kidneys of dogs with long standing hypertension might be limited in the capacity to respond to stimuli which produce a marked increase in renal blood flow in dogs with normal blood pressures. An investigation was therefore made of the renal function of dogs that had maintained consistently elevated blood pressure levels for several years after partial constriction of the renal arteries (1), or section of the carotid sinus and aortic depressor nerves (2). The studies included the measurement of the variations in the clearance of urea under circumstances of widely varying protein intake.

The urea clearance of normal dogs was found by Jolliffe and Smith (3) to rise as much as 100 per cent when the dietary protein was increased from 9.6 to 180 grams per day. This observation was confirmed by Van Slyke, Rhoads, Hiller and Alving (4), who demonstrated further that the variations in the urea clearance were accompanied by parallel changes in the renal blood flow. This phenomenon was utilized to determine the potential capacity of the renal circulation in our chronically hypertensive dogs.

METHODS

Five dogs were used in these experiments. The femoral arterial pressure was measured directly by the method of Hamilton, Brewer and Brotman (5). One

[1] Supported by a grant from the Commonwealth Fund for the Study of Essential Hypertension.

[2] A preliminary report of this work appeared in abstract in the Bulletin of the Johns Hopkins Hospital, **67:** 407, 1940.

[3] Bull. Johns Hopkins Hospital, **72:** 203, 1943.

of the animals, which served as a control, was a normal female (No. 49) in which no surgical procedures were performed and in which the blood pressure remained normal throughout the period of observation. Two dogs (Nos. 3 and 5) had chronic hypertension following partial compression of both renal arteries by the method of Goldblatt, Lynch, Hanzal and Summerville (1). The remaining two dogs (Nos. 15 and 27) had chronic hypertension produced by section of the four moderator (two carotid sinus and two aortic depressor) nerves by the method of Heymans (2, 6). Following the stated operations, each of the four dogs showed persistent elevation of both the systolic and diastolic pressure levels throughout the subsequent years of life, during which they remained in good health and under constant observation. In 1942, all four dogs were killed and autopsies were performed.[4]

Dog 3. Male, weight 11.5 kg. during urea clearance studies, 9 kg. at autopsy. The right renal artery was partially occluded on August 28, 1936, and the left one on September 9, 1936. Well marked hypertension appeared within two weeks which persisted for five years and four months until the dog was killed on January 13, 1942. At autopsy the clamp was on the left main renal artery; the left kidney was atrophic and weighed 2.3 grams. There was extensive collateral circulation to that kidney which suggests that atrophy occurred some time after clamping. The right clamp was found to be on one of the two main branches of the renal artery; the other branch was patent and larger than normal. The left kidney weighed 38.5 grams. The heart weighed 88 grams. The left adrenal gland weighed 1.6 grams, the right adrenal gland 1 gram. On microscopical examination the right renal parenchyma appeared normal. None of the arterioles in any tissue showed thickening or hyalinization. There were no other significant positive findings.

Dog 5. Male, weight 17 kg. during urea clearance studies, 15.7 kg. at autopsy. The right renal artery was partially occluded on December 2, 1936; within two weeks well marked hypertension developed. The left renal artery was partially occluded on March 20, 1937. No increase in the degree of hypertension developed, so the left clamp was tightened on April 14, 1937. The hypertension then increased and persisted for a little over five years until the dog was killed on January 17, 1942. At autopsy both clamps were on the main renal arteries. The left kidney weighed 49.6 grams, the right kidney 48.9 grams. Collateral circulation to both kidneys was extensive. The heart weighed 153.3 grams. The left adrenal gland weighed 1 gram, the right adrenal gland 1.1 gram. On microscopical examination the renal parenchyma and the arterioles throughout the body appeared normal. There were no other significant positive findings.

Dog 15. Male, weight 13 kg. during urea clearance studies, 12 kg. at autopsy. The carotid sinus and aortic depressor nerves on the right side were severed on June 19, 1937. Similar nerve section was performed on the left side on July 6, 1937. Hypertension developed within a few days and persisted for four years and

[4] The authors are indebted to Dr. Sam S. Blackman, Jr., of the Department of Pathology, for his assistance with the pathological examinations.

eight months, until the dog was killed on March 31, 1942. At autopsy the left kidney weighed 41 grams, the right kidney 36 grams. The heart weighed 93 grams. Each adrenal gland weighed 1 gram. On microscopical examination the kidneys and arterioles appeared normal.[5]

Dog 27. Female, weight 12 kg. during urea clearance studies and at autopsy. The four moderator nerves were each sectioned separately between July 13 and August 12, 1938. Severe hypertension developed within a few days and persisted for three years and nine months, until the dog was killed on April 9, 1942. At autopsy the left kidney weighed 28 grams, the right kidney 27 grams. The heart weighed 91 grams. Each adrenal gland weighed 1 gram. On microscopical examination the kidneys and arterioles appeared normal.

During the low protein studies the dogs were fed the diet of Jolliffe and Smith (3), consisting of 100 grams of cracker meal, 30 grams of lard and 30 grams of cane sugar per day. One tablespoonful of cod liver oil and of brewer's yeast and one gram of salt mixture were added. The calculated total protein content was approximately 15 grams. The high protein diet consisted of 2 pounds of sliced pig heart per day, containing approximately 150 grams of protein. The dogs were maintained on the appropriate diet for at least seven days prior to the beginning of a series of observations. Each low protein diet experiment was made after a fast of 18 hours, but during the high protein diet studies, the dogs were fed 2 pounds of meat one to two hours before each experiment.

Urea clearance determinations were also made, in the hypertensive animals, while they were taking a normal, mixed diet[6] which contained approximately 40 grams of protein per daily ration.

In the dogs with renal hypertension, 0.5 grams of urea per kilo, plus 12.5 grams of sucrose, made up as a 50 per cent solution, were injected intravenously 15–20 minutes before the start of each experiment, to insure an adequate urine flow and to raise the blood and urine urea content to levels which would reduce analytical errors to a minimum. In the dogs with neurogenic hypertension, 0.5 gm. of urea per kilo in 300 cc. of water was given by stomach tube 30 minutes before the experiment was begun, but no sucrose or urea was given intravenously. These two methods were found to be equally effective and did not produce any detectable differences in the urea clearances. Urine was collected through an inlying catheter, each period being terminated by bladder lavage. Blood samples were drawn from the jugular vein at the precise mid-point of each clearance period.

Blood and urine urea determinations were performed by the alkaline hypobromite method, as applied to manometric analysis by Van Slyke (7). The clearances were calculated from the formula $\frac{UV}{BSa}$, where U = urine urea N content in mgm. per cc.; V = total volume of urine plus wash-out, as cc. per minute; B = blood urea N content in mgm. per cc.; Sa = the surface area in square meters,

[5] Detailed studies of the pathological findings in dogs with neurogenic hypertension will be published later by one of us, (C. B. T.).

[6] One pound "Silver Klass" Dog Food (Esskay Co., Baltimore) per dog per day.

calculated from the formula of Cowgill and Drabkin, as modified by Rhoads, Alving, Hiller and Van Slyke (8). The weights of the kidneys were determined at post mortem.

RESULTS

In all the dogs there was a marked rise in the urea clearance values with the change from the low to the high protein intake (Tables I–III, fig. 1). There were quantitative differences in the responses of the various dogs, but in view of the small series of animals used, it is impossible to evaluate these precisely. The clearances measured during the normal mixed diets were slightly higher than those made while the dogs received the low protein diet.

TABLE I

Urea clearances in normal dog while consuming low and high protein diets

Dog #49. Female. Surface area, 0.525 square meters. Total kidney weight, 63.0 grams.

DIET	DATE	UREA CLEARANCE	
		cc./min./M²	cc./min./gm. kidney
Low Protein	Oct. 15, 1941	64.7	0.539
		54.2	0.451
		54.1	0.450
	Oct. 17, 1941	52.6	0.438
		39.5	0.328
		45.0	0.375
		46.7	0.389
Mean		50.9	0.424
High Protein	Dec. 15, 1941	76.7	0.639
		75.3	0.627
		90.4	0.752
		98.3	0.819
	Jan. 21, 1942	97.1	0.810
		93.7	0.782
		89.1	0.743
		96.0	0.800
Mean		89.6	0.746

Ratio of mean urea clearance on high protein diet/low protein diet = 1.76.

TABLE II

Urea clearances in renal hypertensive dogs while receiving low and high protein diets

DIET	DOG NO. 3. MALE. SURFACE AREA, 0.460 SQUARE METERS. TOTAL KIDNEY WEIGHT, 40.8 GRAMS			DOG NO. 5. MALE. SURFACE AREA, 0.695 SQUARE METERS. TOTAL KIDNEY WEIGHT, 98.5 GRAMS		
	Urea clearance			Urea clearance		
	Date	cc./min./M^2	cc./min./gm. kidney	Date	cc./min./M^2	cc./min./gm. kidney
Low protein	Mar. 4, 1940	33.7	0.380	Feb. 12, 1940	47.8	0.337
		34.9	0.394		45.2	0.318
		37.3	0.421		45.2	0.319
	Mar. 6, 1940	26.0	0.293	Feb. 14, 1940	47.6	0.335
		25.5	0.288		38.7	0.272
		28.7	0.327		40.0	0.282
	Mar. 8, 1940	29.6	0.334	Feb. 16, 1940	42.4	0.299
		28.5	0.321		41.3	0.291
		40.6	0.458		39.9	0.281
Mean		31.6	0.357		43.1	0.304
High protein	Mar. 18, 1940	82.9	0.935	Feb. 27, 1940	106.3	0.750
		56.7	0.640		106.0	0.748
		64.4	0.725		87.3	0.616
	Mar. 20, 1940	89.0	1.004	Feb. 28, 1940	103.4	0.729
		82.9	0.935		109.0	0.769
		78.6	0.885		98.9	0.697
	Mar. 21, 1940	87.9	0.991	Mar. 1, 1940	113.2	0.789
		83.6	0.942		120.3	0.849
		86.2	0.972		112.0	0.790
Mean		79.1	0.892		106.2	0.748
Mixed	Feb. 20–23, 1940	42.3		Jan. 18–Feb. 2, 1940	53.7	
Ratio of mean urea clearance high protein/low protein diet	2.50			2.46		

The blood pressure readings are shown in fig. 2. In the sections designated as Normal Diet are shown the ranges of the highest and lowest

TABLE III

Urea clearances in neurogenic hypertensive dogs while consuming low and high protein diets

DIET	DOG NO. 15. MALE. SURFACE AREA, 0.594 SQUARE METERS. TOTAL KIDNEY WEIGHT, 77.0 GRAMS			DOG NO. 27. FEMALE. SURFACE AREA, 0.558 SQUARE METERS. TOTAL KIDNEY WEIGHT 55.0 GRAMS		
	Urea clearance			Urea clearance		
	Date	cc./min./M²	cc./min./gm. kidney	Date	cc./min./M²	cc./min./gm. kidney
Low protein	April 22, 1940	49.7	0.383	Mar. 24, 1940	56.3	0.571
		54.4	0.419		61.8	0.627
		59.7	0.461		54.8	0.555
	April 24, 1940	51.5	0.397	Mar. 25, 1940	58.9	0.597
		55.5	0.428		62.5	0.634
		54.7	0.422		66.7	0.677
	April 26, 1940	56.9	0.438	Mar. 27, 1940	53.3	0.451
		61.7	0.476		61.1	0.620
		63.2	0.487		61.9	0.628
Mean		56.3	0.434		59.7	0.606
High protein	May 3, 1940	110.8	0.854	April 15, 1940	106.3	1.076
		101.4	0.782		134.7	1.365
		85.4	0.689		112.5	1.141
	May 9, 1940	151.2	1.167	April 17, 1940	83.7	0.850
		135.0	1.040		100.3	1.018
		115.5	0.891		91.8	0.931
	May 13, 1940	135.3	1.045	April 19, 1940	133.7	1.356
		116.9	0.902		109.0	1.105
		111.0	0.856		101.0	1.024
Mean		118.0	0.910		108.1	1.096
Mixed	April 8–12, 1940	74.5		Mar. 11–15, 1940	70.5	
Ratio of mean urea clearance high protein/low protein diet	2.09			1.81		

systolic and diastolic pressures obtained during the year preceding the present studies. During the experimental periods the blood pressure

determinations were made after at least one week on the appropriate special diet. No change in the pressure levels which could be interpreted as an effect of the diet appeared in the group as a whole, and the individual readings, with a few exceptions, were within the range previously observed when the dogs had received the normal diet.

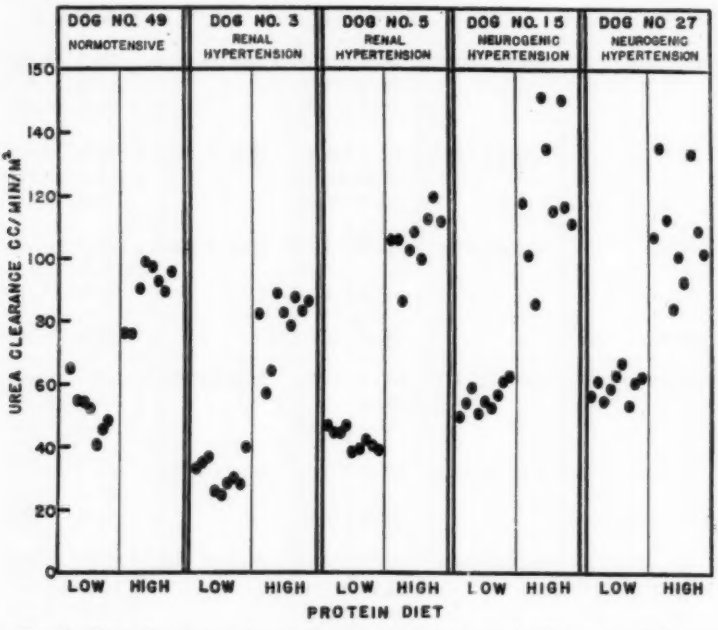

FIG. 1. Urea clearance values during low and high protein diets in normal, renal hypertensive and neurogenic hypertensive dogs.

DISCUSSION

The relation between dietary protein, urea clearance and renal blood flow

The effect of meat in raising the urea clearance in normal dogs was observed by Shannon, Jolliffe and Smith (9) to occur in the course of four or five hours following a single meal, but a maximum effect was obtained after feeding meat when it was also used in the maintenance diet. Casein,

FIG. 2. Systolic (solid dots) and diastolic (open circles) blood pressure readings in normal, renal hypertensive and neurogenic hypertensive dogs while receiving normal, low protein and high protein diets. In the hypertensive dogs, under the headings labelled Normal Diet the extreme range of variation in the blood pressure readings during the year immediately preceding the beginning of the present experiments is represented by solid and open rectangles to denote systolic and diastolic blood pressures, respectively.

and the amino acids glycine, glycylglycine and alanine individually affect the urea clearance in a manner similar to meat, according to Pitt (10).

Van Slyke, Rhoads, Hiller and Alving (4), using dogs with explanted kidneys, noted that the variations in the urea clearance, which are associated with the alterations in diet, were not due to changes in the percentage of urea extracted from the blood by the kidneys but to variations in renal blood flow. The increase in renal circulation following the ingestion of protein may be part of a rise in general circulation rate, since, as Herrick, Essex, Mann and Baldes (11) have observed, the blood flow in the femoral, carotid and mesenteric arteries is increased after feeding. Pitts (10) injected thyroxin into a dog and noted a rise in the urea clearance in 24 hours, which he attributed to an increased breakdown of endogenous protein. However, in view of the known effect of thyroxin in raising the cardiac output and general circulation rate, the change in the urea clearance may have been associated to some extent with the rise in renal blood flow due to the general cardiovascular effects.

In man, variations in protein intake have been found by several investigators to produce changes in the clearance of urea. Keutmann and McCann (12), were able to raise the urea clearances of patients with Bright's disease between 50 and 87 per cent by changing from low to high protein diets. Cope (13), also using patients with nephritis, obtained an average increase of only 40 per cent in the subjects with normal or nearly normal renal function, and much less effect in those with reduced kidney function. Goldring, Raginsky, Greenblatt and Cohen (14) noted that in normal men a change from a low protein to a normal diet raised the urea clearance by 15 to 50 per cent, but that additional protein did not elevate the clearance any further. Duel, Sandiford, Sandiford and Boothby (15) were able to maintain a subject on a low protein intake for a prolonged period, and from their figures Shannon et al. (9) calculated that after 32 days the urea clearance fell from an initial value of 53.4 to a final one of 9.6 cc. per minute. There was no evidence of renal insufficiency in their subject at any time. In four children with nephrosis, Farr (16) observed that, by varying the protein intake from 0.5 to 4 grams per kilo, the urea clearance was increased to an extent varying from 134 per cent to 195 per cent. It seems, therefore, that the effect of dietary protein on the urea clearance of adult men is less pronounced than it is in dogs, but in children the response is much greater.

Kidney function in chronic hypertension of renal origin

Goldblatt and his co-workers (1) determined the urea clearance values before and after hypertension developed in their dogs. Among the animals with marked constriction of the renal arteries, two developed a pronounced fall in the urea clearance and died in uremia within a short time, while those that survived for several weeks had a persistent reduction in clearance of about 50 per cent. The dogs in which the renal arteries were only moderately constricted survived for variable periods up to 15 months with consistently normal urea clearances.

Corcoran and Page (17) reported that they were able to induce a persistent hypertension in dogs without any alteration in the urea clearance.

In the two dogs which we have described with renal hypertension of 4 and 5 years duration, the urea clearance values were normal when the animals received the low protein diet, and were increased by 150 per cent during the period of high protein intake.

Renal function in experimental neurogenic hypertension

In dogs which had persistent hypertension for two and a half years after section of the carotid sinus and aortic depressor nerves, Hoerner, Fontaine and Mandel (18) found no reduction in the Ambard constants or rates of excretion of phenolsulphonephthalein. They also noted that histological sections of the kidneys revealed no structural abnormalities.

In the two dogs reported here with neurogenic hypertension of three and four years' duration, the urea clearance values when they consumed the low protein diet were normal. When the high protein diet was taken the urea clearance was increased, on the average, by 95 per cent. The variations in the individual clearance determinations during the period of high protein diet were greater in these dogs than in the renal hypertensive animals. This may have been associated with the vascular instability which is characteristic of neurogenic hypertensive dogs and is reflected in the wide fluctuations in their blood pressure readings.

The relation between experimental hypertension and dietary protein

The influence of variations in the intake of protein on the blood pressure of animals with renal hypertension was first studied by Verney and Vogt (19). They found that a diet of meat alone produced only slight

and variable changes in pressure, whereas a large intake of salt or urea was accompanied by a rise in blood pressure. Machlachlen and Taylor (20) reported an acute rise in the blood pressure of hypertensive dogs after the ingestion of meat or urea. Cash and Wood (21), on the other hand, noted, in the one hypertensive dog which they studied, that a rise in blood pressure on a high meat diet occurred only when the dog gained weight. Philipsborn, Katz and Rodbard (22) observed that high protein diets had no effect on the blood pressure of their hypertensive animals when the renal function was normal. When renal excretory insufficiency was present, two of their dogs showed a rise in arterial pressure during the period of high meat intake, while eight dogs did not.

In our animals (fig. 2) the blood pressure readings over a long period on normal diets showed wide fluctuations, in spite of the fact that the dogs were well trained and the circumstances surrounding each arterial puncture were kept the same so far as possible. If changes in blood pressure during special diets are to be considered significant, they should deviate beyond the previously observed range or should show a consistent tendency in one direction or the other. From our observations neither of these criteria were satisfied.

CONCLUSIONS

If the parallelism between the variations in the urea clearance and the renal blood flow observed by Van Slyke et al. (4) in normal dogs can be applied to the results described in this paper, the conclusion appears justified that the vascular supply to the kidneys of dogs with long standing hypertension, either of renal or neurogenic origin, is undiminished in size or activity.

It also appears that changes in dietary protein have no detectable influence on the blood pressure levels of dogs with chronic experimental hypertension.

SUMMARY

1. Determinations of the clearance of urea during periods of varying protein intake were made in five dogs. Two of the dogs had chronic experimental hypertension of renal origin, two had chronic neurogenic hypertension, and one had a normal blood pressure.

2. The urea clearance values were normal in all of the animals during periods of low protein intake and showed pronounced elevations when the dogs were fed diets high in protein.

3. Since variations in urea clearance are associated with parallel changes in renal blood flow, these results suggest that there may be no impairment of renal blood flow in dogs with chronic experimental hypertension.

4. The variations in arterial pressure during the experimental diets were not great, and could not be correlated with the changes in protein intake or urea clearance.

BIBLIOGRAPHY

1. Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W.: *J. Exp. Med.*, **59:** 347, 1934.
2. Heymans, C.: *Compt. rend. Soc. de biol.*, **107:** 1293, 1931.
3. Jolliffe, N., and Smith, H. W.: *J. Physiol.*, **99:** 101, 1931.
4. Van Slyke, D. D., Rhoads, C. P., Hiller, A., and Alving, A.: *Am. J. Physiol.*, **110:** 387, 1934.
5. Hamilton, W. F., Brewer, G., and Brotman, I.: *Am. J. Physiol.*, **107:** 427, 1934.
6. Thomas, C. B., and Warthin, T. A.: *Am. Heart J.*, **19:** 316, 1940.
7. Van Slyke, D. D.: *J. Biol. Chem.*, **83:** 449, 1929.
   Van Slyke, D. D., and Kugel, V. H.: *J. Biol. Chem.*, **102:** 489, 1933.
   Van Slyke, D. D., Page, I. H., Hiller, A., and Kirk, E.: *J. Clin. Invest.*, **14:** 901, 1935.
8. Rhoads, C. P., Alving, A. S., Hiller, A., and Van Slyke, D. D.: *Am. J. Physiol.*, **109:** 329, 1934.
9. Shannon, J. A., Jolliffe, N., and Smith, N. W.: *Am. J. Physiol.*, **101:** 625, 1932.
10. Pitts, R. F.: *J. Nutrition*, **9:** 657, 1935.
11. Herrick, J. F., Essex, H. E., Mann, F. C., and Baldes, E. J.: *Am. J. Physiol.*, **108:** 621, 1934.
12. Keutmann, E. H., and McCann, W. S.: *J. Clin. Invest.*, **11:** 973, 1932.
13. Cope, C. L.: *J. Clin. Invest.*, **12:** 567, 1933.
14. Goldring, W., Raginsky, L., Greenblatt, M., and Cohen, S.: *J. Clin. Invest.*, **13:** 743, 1934.
15. Duel, H. J., Jr., Sandiford, I., Sandiford, K., and Boothby, W. M.: *J. Biol. Chem.*, **76:** 391, 1928.
16. Farr, L. E.: *J. Clin. Invest.*, **15:** 703, 1936.
17. Corcoran, A. C., and Page, I. H.: *Am. J. Physiol.*, **123:** 43, 1938.
18. Hoerner, G., Fontaine, R., and Mandel, P.: *Arch. d. Mal. du Coeur*, **64:** 1090, 1938.
19. Verney, E. B., and Vogt, M.: *Quart. J. Exp. Physiol.*, **28:** 253, 1938.
20. Maclachlen, I., and Taylor, N. B.: *Am. J. Physiol.*, **129:** 413, 1940.
21. Cash, J. R., and Wood, J. E., Jr.: *South. Med. J.*, **31:** 270, 1938.
22. Philipsborn, H., Katz, L. N., and Rodbard, S.: *J. Exp. Med.*, **74:** 591, 1941.

STUDIES IN EXPERIMENTAL HYPERTENSION[1]

III. The Effect of Dietary Protein on the Clearances of Diodrast and Inulin by the Kidney in Chronic Hypertension

LOUIS K. ALPERT, M.D., AND JOSEPH L. LILIENTHAL, JR., M.D.

From the Department of Medicine, Johns Hopkins University School of Medicine

Received for publication January 26, 1943

In view of the fact that the excretion of urea by the kidney is dependent upon both glomerular and tubular functions, the investigation of the response of normal and chronically hypertensive dogs to changes in dietary protein was extended to include determinations of the clearances of inulin and diodrast. The inulin clearance was established as a measure of glomerular filtration by Richards, Westfall and Bott (1) and by Shannon (2), and Shannon and Smith (3). The excretion of diodrast was demonstrated by Elsom, Bott and Shiels (4) and by Smith, Goldring and Chasis (5) to take place largely by the renal tubules. The latter authors have also indicated that since the clearance of diodrast at low plasma concentrations is greater than that of any other known substance, it may be utilized as a measure of the renal plasma flow and, by appropriate calculation, of the "effective" total blood flow through the human kidney. In dogs, however, White and Heinbecker (6) and Corcoran, Smith and Page (7) observed that the plasma clearance of diodrast was less than the renal plasma flow by 13 to 17 per cent. Nevertheless, since our experiments were concerned primarily with comparative values during consumption of low and high protein diets, we felt justified in employing the diodrast clearance to measure the changes in renal blood flow with the expectation that it would offer a greater degree of accuracy than was afforded by the urea clearance.

METHODS

The dogs and diets used in these experiments were identical with those described in the preceding paper.[2] One additional normal female (No. 50) was included. This dog had difficulty in consuming as much meat as the others did, and her aver-

[1] Supported by a grant from the Commonwealth Fund for the Study of Experimental Hypertension.

[2] The hypertensive dogs used were part of a group originally prepared and studied by Dr. Caroline B. Thomas, which were kindly loaned to us for these experiments.

age intake on the high protein diet was approximately three-fourths to one pound of meat per day, with about one-half pound in addition one hour before each high protein diet experiment. The resultant changes in the clearances were less striking but similar to those of the other dogs who consumed two pounds of meat per day during the period of high protein diet.

The determinations of inulin in plasma and urine were performed according to the method of Alving, Rubin and Miller (8), with modifications recommended by them (personal communication) and by Goldring, Chasis, Ranges and Smith (9). Diodrast iodine was measured by the method previously described (10). Urea was estimated by the hypobromite method, as in the preceding paper.

The plan of each experiment was as follows. The dog was given a liter of tap water in divided portions by stomach tube, and an inlying catheter was placed in the bladder. A venous blood sample was drawn for determinations of inulin plasma "blank" values. When a urine flow of more than one cc. per minute appeared to have been established, a priming dose of 3 to 5 cc. of 10 per cent inulin and 1 cc. of 35 per cent diodrast[3] was injected intravenously, followed at once by a continuous infusion of normal saline containing approximately 6 cc. of 35 per cent diodrast and 30 cc. of 10 per cent inulin per liter. No urea was administered. The rate of the infusion was maintained by a calibrated drip bulb at 2.5 cc. per minute. These figures were varied somewhat, according to the size of the dog, in order to maintain a plasma level of one to two milligrams per cent of diodrast iodine and 10 to 15 milligrams per cent of inulin. After allowing 15 to 20 minutes for stabilization of the infusion inflow and blood concentrations, a sample of venous blood was drawn. Then 4 or 5 urine collection periods were carried out, each of 10 to 15 minutes duration, including terminal lavage of the bladder. Additional blood samples were drawn between the second and third and after the final urine collections. All times were accurately noted.

Calculations

The observed plasma concentrations of diodrast iodine, inulin and urea were plotted with respect to the time of collection of the blood on semi-logarithmic paper. The periods of urine collection were also plotted on the same time scale. The plasma values at the mid-point of each period were then determined by interpolation. The urine flows were estimated by subtracting the amount of wash water used, and the urine concentrations were then corrected by the appropriate dilution factors. The clearance values were calculated by the formula $\frac{UV}{BSa}$, as described in the preceding paper.

RESULTS

From the data which are presented in tables I to VII and in figures 1 and 2, it is apparent that the change from a low to a high protein diet

[3] Ampoules of sterile 35 per cent Diodrast solution were kindly supplied by the Winthrop Chemical Co., New York.

was accompanied in all the dogs by an increase in the clearances of diodrast and inulin as well as of urea. The relative magnitude of these changes, however, was not the same for all the substances under consideration. The average increase in the inulin clearances was 118 per cent, in the urea clearances, 90 per cent, and in the diodrast clearances 68 per

TABLE I

Diodrast, Inulin and Urea Clearances in Normal Dog while Taking Low and High Protein Diets

Dog no. 49. Female. Surface area 0.525 square meters

DATE	DIET	URINE FLOW	DIODRAST IODINE PLASMA CONC.	PLASMA CLEARANCE (CC./MIN./M²)		
				Diodrast	Inulin	Urea
		cc./min.	*mgm. per cent*			
Oct. 15, 1941	Low protein	0.26	2.11	192.2	79.3	31.4
		0.60	1.98	212.5	79.3	64.7
		1.56	1.85	217.0	77.5	54.2
		1.93	1.71	246.0	76.7	54.1
Oct. 17, 1941	Low protein	4.87	1.80	224.0	83.9	52.6
		2.10	1.67	194.0	72.7	39.5
		1.80	1.63	225.0	77.5	45.0
		6.87	1.65	206.8	79.1	46.7
Mean				214.7	78.2	48.5
Dec. 15, 1941	High protein	1.75	1.24	319.0	197.5	76.7
		1.61	1.22	312.8	185.0	75.3
		1.81	1.20	431.5	178.3	90.4
		1.26	1.17	394.0	201.4	98.3
Jan. 21, 1942	High protein	2.47	0.94	303.0	126.2	97.1
		2.93	0.86	299.5	111.5	93.7
		2.54	0.79	277.4	131.5	89.1
		1.33	0.74	326.0	141.0	96.0
Mean				332.9	159.0	89.5

cent. No significant distinction could be made between the responses of the renal hypertensive dogs and those of the neurogenic hypertensive animals. The relatively smaller changes which occurred in the normal dogs may be attributed to the fact that they consumed less meat during the high protein diet periods than the hypertensive ones did.

It is noteworthy that wide fluctuations were observed in the individual diodrast and inulin clearances, and to a lesser extent in the urea clearances, during the period of high protein intake. This was most marked in the neurogenic hypertensive dogs. These variations could not be correlated with changes in urine flow from one experiment to another,

TABLE II

Diodrast, inulin and urea clearances in normal dog while taking low and high protein diets

Dog no. 50. Female. Surface area 0.577 square meters

DATE	DIET	URINE FLOW	DIODRAST IODINE PLASMA CONC.	PLASMA CLEARANCE (CC./MIN./M²)		
				Diodrast	Inulin	Urea
		cc./min.	*mgm. per cent*			
Sept. 3, 1941	Low protein	4.60	1.63	215.8	84.2	51.1
		5.30	1.50	225.5	81.3	52.0
		5.40	1.40	226.0	87.1	52.5
		4.75	1.30	215.4	89.0	47.0
		3.70	1.28	242.8	94.2	55.1
Sept. 5, 1941	Low protein	6.70	1.87	217.5	75.4	50.1
		7.90	1.73	213.8	80.4	55.6
		7.40	1.60	205.0	84.4	53.7
		7.08	1.49	224.0	80.5	53.2
		5.60	1.47	220.0	78.2	46.4
Mean				220.6	83.5	51.7
Dec. 12, 1941	High protein	3.20	1.41	279.0	129.4	
		5.00	1.42	303.3	136.5	
		4.47	1.43	265.5	126.2	
Jan. 23, 1942	High protein	0.40	1.54	254.5	110.0	61.5
		0.60	1.21	271.5	129.0	69.8
		0.88	0.99	319.0	148.2	75.2
		1.00	0.96	282.5	147.6	74.2
Mean				282.0	132.4	70.2

although within each experiment there was a tendency for the clearances to vary in the same direction as the urinary output. It seems more likely that these variations reflect the fluctuations in the renal blood flow, as will be discussed below. In the neurogenic hypertensive dogs, they may be considered as a further manifestation of the vasomotor instability which is characteristic of these animals.

TABLE III

Diodrast, inulin and urea clearances in renal hypertensive dog while taking low and high protein diets

Dog no. 3. Male. Surface area 0.460 square meters

DATE	DIET	URINE FLOW	DIODRAST IODINE PLASMA CONC.	PLASMA CLEARANCE (CC./MIN./M²)		
				Diodrast	Inulin	Urea
		cc./min.	*mgm. per cent*			
Feb. 28, 1941	Low protein	1.93	3.00	143.0	62.0	
		1.62	2.47	148.0	61.5	
		1.58	2.06	146.8	64.1	
		1.21	1.63	184.6	57.6	
Mar. 7, 1941	Low protein	0.42	3.62	111.9	68.6	
		0.38	1.97	172.5	74.1	
		1.11	1.67	177.7	72.2	
		1.41	1.45	164.2	45.6	
June 30, 1941	Low protein	3.00	1.12	217.0	60.6	32.3
		3.85	1.09	180.6	53.5	42.5
		2.22	1.07	187.4	60.4	27.8
		2.75	1.07	217.0	76.2	40.0
July 2, 1941	Low protein	4.90	1.63	213.4	67.5	44.5
		4.08	1.51	190.0	62.8	41.1
		3.37	1.35	205.5	64.6	41.5
		2.18	1.20	197.2	73.5	29.3
Mean				178.5	64.0	37.4
July 14, 1941	High protein	5.33	1.03	371.0	133.5	79.5
		5.72	0.94	382.5	160.0	80.2
		5.00	0.86	333.0	178.8	68.7
		4.60	0.78	321.0	166.6	65.0
		4.50	0.75	328.0	181.0	65.4
Sept. 29, 1941	High protein	3.78	3.06	349.5	169.8	86.3
		4.13	2.76	326.0	150.0	82.8
		4.38	2.52	328.8	149.1	79.5
		4.63	2.30	321.1	155.5	80.4
		4.72	2.25	322.5	152.6	81.8
Mean				338.3	160.0	77.0

TABLE IV

Diodrast, inulin and urea clearances in renal hypertensive dog while taking low and high protein diets

Dog no. 5. Male. Surface area 0.695 square meters

DATE	DIET	URINE FLOW	DIODRAST IODINE PLASMA CONC.	PLASMA CLEARANCE (CC./MIN./M²)		
				Diodrast	Inulin	Urea
		cc./min.	*mgm. per cent*			
Feb. 26, 1941	Low protein	3.33	0.97	271.0	71.9	
		3.88	0.84	253.0	81.6	
		3.67	0.72	247.5	80.3	
		3.77	0.65	271.0	80.6	
		2.61	0.59	262.0	86.3	
Mar. 5, 1941	Low protein	2.70	1.64	201.0	92.8	
		2.38	1.17	204.5	83.8	
		3.00	1.03	250.2	100.7	
		2.83	0.99	229.0	99.3	
		2.42	0.96	221.8	97.1	
June 23, 1941	Low protein	0.38	4.05	250.0	62.2	46.5
		0.35	3.50	205.1	74.4	42.9
		0.23	2.90	247.0	93.9	50.1
		0.35	2.70	270.0	103.6	34.9
		0.38	2.52	258.0	111.4	52.0
June 25, 1941	Low protein	3.85	1.87	240.8	64.3	46.4
		4.15	1.73	255.8	69.1	58.8
		4.20	1.68	274.0	88.3	56.3
		2.64	1.65	231.8	72.9	39.8
Mean				244.4	84.9	47.5
July 8, 1941	High protein	6.20	1.53	445.0	222.9	108.2
		3.55	1.45	378.0	236.8	86.5
		3.40	1.39	337.0	173.5	77.4
July 11, 1941	High protein	7.00	1.20	437.5	230.6	106.5
		8.40	1.04	469.0	204.0	100.2
		9.90	0.98	512.0	220.0	112.0
		8.40	0.94	471.0	224.4	113.3
		10.60	0.94	550.0	217.5	115.6
Mean				449.9	216.2	102.4

TABLE V

Diodrast, inulin and urea clearances in neurogenic hypertensive dog while taking low and high protein diets

Dog no. 15. Male. Surface area 0.594 square meters

DATE	DIET	URINE FLOW	DIODRAST IODINE PLASMA CONC.	PLASMA CLEARANCE (CC./MIN./M²)		
				Diodrast	Inulin	Urea
		cc./min.	*mgm. per cent*			
July 21, 1941	Low protein	2.66	1.60	236.2	79.0	46.3
		3.88	1.55	296.5	113.8	72.4
		2.33	1.48	239.8	74.9	46.8
		1.20	1.39	257.2	93.3	55.9
Aug. 25, 1941	Low protein	2.55	1.87	247.5	77.2	46.6
		2.20	1.72	253.5	82.5	46.8
		5.00	1.61	277.3	92.5	59.4
		5.93	1.56	257.2	93.1	58.9
		4.90	1.59	273.1	112.5	57.3
Aug. 27, 1941	Low protein	6.10	1.70	309.5	96.0	64.3
		4.70	1.60	284.5	85.0	58.1
		4.60	1.51	295.5	87.9	60.9
		4.10	1.43	328.5	90.0	60.3
		4.10	1.38	316.0	74.9	58.6
Mean				276.6	89.4	56.6
Sept. 10, 1941	High protein	1.32	1.22	469.0	230.0	105.2
		3.70	1.15	608.0	233.2	147.3
		4.40	1.10	445.5	176.8	106.8
		6.70	1.06	461.0	198.5	107.9
		8.20	1.06	426.0	226.0	109.6
Sept. 12, 1941	High protein	2.11	0.97	508.0	221.9	128.0
		1.68	0.95	578.0	242.4	140.5
		2.55	0.93	633.0	293.0	164.3
		2.35	0.90	484.5	212.0	134.8
Mean				512.5	226.0	127.1

The effect of diet on the urea clearance/inulin clearance ratio

The magnitude of the rise which occurred in the inulin clearance of the normal as well as the hypertensive dogs indicates that the increase in glomerular filtration was the dominant factor in the augmentation of

TABLE VI

Diodrast, inulin and urea clearances in neurogenic hypertensive dog while taking low and high protein diet

Dog no. 27. Female. Surface area 0.558 square meters

DATE	DIET	URINE FLOW	DIODRAST IODINE PLASMA CONC.	PLASMA CLEARANCE (CC./MIN./M²)		
				Diodrast	Inulin	Urea
		cc./min.	*mgm. per cent*			
July 28, 1941	Low protein	4.46	1.78	255.8	83.9	67.2
		3.09	1.72	262.5	96.9	60.9
		1.65	1.70	215.5	78.7	49.3
		0.44	1.58	240.5	86.0	52.1
July 30, 1941	Low protein	2.70	1.84	261.0	99.1	78.8
		2.30	1.72	259.5	96.2	63.8
		2.10	1.59	314.0	118.3	75.3
		1.90	1.48	315.0	115.6	73.5
		1.30	1.44	304.0	114.1	68.5
Mean				269.7	98.7	65.5
Aug. 13, 1941	High protein	4.00	1.69	371.0	144.2	111.1
		2.50	1.52	337.5	135.3	105.8
		2.78	1.35	394.5	157.1	122.8
		2.38	1.22	457.5	160.1	127.7
		1.00	1.09	387.5	156.0	94.4
Aug. 15, 1941	High protein	1.60	1.38	411.0	214.5	108.5
		4.21	1.19	607.0	299.5	162.5
		1.66	1.14	533.0	256.0	130.3
		4.50	1.09	555.0	325.0	152.0
		5.50	1.06	521.0	265.2	132.5
Sept. 24, 1941	High protein	5.77	1.27	424 0	140.5	120.5
		6.70	1.28	383.0	133.3	106.7
		6.80	1.30	418.5	181.5	116.7
		3.70	1.31	391.5	143.5	103.1
		1.10	1.33	525.0	126.2	94.1
Mean				447.7	189.2	119.2

the urea clearance in these animals. This confirms the observations of Jolliffe and Smith (11) and Shannon, Jolliffe and Smith (12) in normal dogs. The relative increase in the clearance of urea, however, was gen-

erally less than that in the inulin clearance. Hence the ratio of the urea clearance to the inulin clearance was somewhat lower during the period of high protein diet than it was on the low protein intake. Since the rate of filtration of urea by the glomeruli should be the same as that of inulin, the smaller rise in the total excretion of urea by the kidneys would indicate a greater reabsorption of urea by the tubules during the period of high protein diet. However, in view of the wide fluctuations in the individual clearance values, the relatively small average differences between the changes in the urea and inulin clearances may, as Shannon,

TABLE VII

Summary of data in normal, renal hypertensive and neurogenic hypertensive dogs while taking low and high protein diets

DOG NO.	HYPERTENSION	DIETARY PROTEIN	MEAN PLASMA CLEARANCES (cc./MIN./M²)			RATIO C_I/C_D	RATIO C_U/C_I	RATIO OF CLEARANCES ON HIGH PROTEIN DIET/ LOW PROTEIN DIET		
			Diodrast	Inulin	Urea			Diodrast	Inulin	Urea
49	None	Low	214	78	48	0.36	0.62	1.55	2.04	1.86
		High	333	159	89	0.48	0.56			
50	None	Low	220	83	52	0.38	0.63	1.28	1.59	1.35
		High	282	132	70	0.47	0.53			
3	Renal	Low	179	64	37	0.36	0.58	1.89	2.50	2.08
		High	338	160	77	0.47	0.48			
5	Renal	Low	244	85	48	0.35	0.56	1.84	2.54	2.12
		High	450	216	102	0.48	0.47			
15	Neurogenic	Low	277	89	57	0.32	0.64	1.85	2.54	2.22
		High	513	226	127	0.44	0.56			
27	Neurogenic	Low	270	99	66	0.37	0.67	1.66	1.91	1.80
			448	189	119	0.42	0.63			

et al. (12) have suggested, be within the range of experimental variation. It seems clear, however, that no decrease in tubular reabsorption of urea took place, which might have contributed to its increased excretion when the high protein diet was consumed.

The effect of diet on the diodrast clearance

The efficiency of the kidney in excreting diodrast has been shown by other investigators to be at its maximum when the plasma concentration of diodrast is low, and only under those circumstances does the plasma diodrast clearance approximate the renal plasma flow. Hence, in all

our experiments, the plasma concentrations of diodrast iodine were maintained at levels below 5 milligrams per cent.

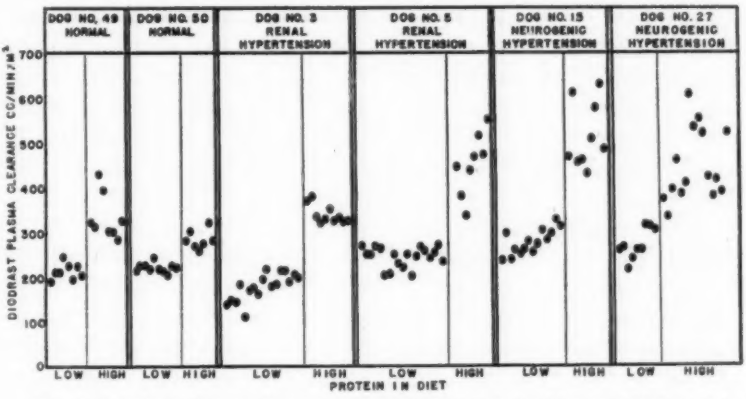

FIG. 1. Diodrast plasma clearance values during periods of low and high protein diets in normal, renal hypertensive and neurogenic hypertensive dogs.

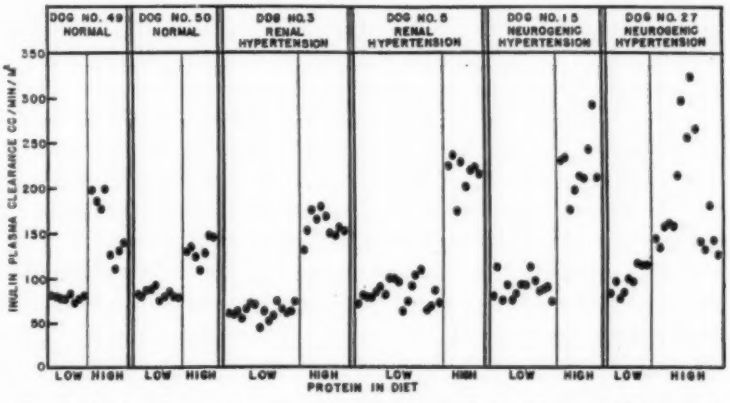

FIG. 2. Inulin plasma clearance values during periods of low and high protein diets in normal, renal hypertensive and neurogenic hypertensive dogs.

The increase in the diodrast clearance which occurred in all the dogs when a change was made from the low to the high protein diet, may be interpreted as due either to an improvement in the capacity of the tubular

cells to excrete this substance, or to a rise in the renal blood flow, which would bring more diodrast to the tubules for excretion. The relationship between the changes in the urea and inulin clearances, which was discussed above, indicates that any change in tubular activity which may have taken place was not great. This is also indicated by White's observations (6) in normal dogs with explanted kidneys. He found that an average of 73 per cent of the renal arterial plasma diodrast was removed by the kidneys. Corcoran, Smith and Page (7), in similar experiments obtained a mean extraction figure of 85 per cent. Hence, according to these observations, between 15 and 27 per cent of the arterial diodrast remains unextracted by the kidney. These figures have been confirmed by recent experiments of our own. If these observations are applied to the experiments described here, then the maximum rise in the diodrast clearance which may be expected from increased extraction alone is 15 to 27 per cent. Since the actual rise in diodrast clearance which occurred with the change from the low to the high protein diets ranged from 28 per cent to 89 per cent (Table VII), it is apparent that greater renal extraction of diodrast is not adequate to account for the changes observed. It appears justifiable to conclude, therefore, that the variations which were observed in the diodrast clearance of both the normal and the hypertensive dogs were due to changes in renal blood flow.[4]

The ratio between the inulin and diodrast clearance during the period of low protein diet was higher in these dogs than the average figure of 0.20 which has been found in normal men. This discrepancy is probably due to the quantitative deficiency of the diodrast clearance as an absolute measure of renal plasma flow in dogs, as compared with men. Nevertheless, it is noteworthy that this ratio became increased with the change to the high protein diet as a result of the larger rise in the inulin than in the diodrast clearances. In terms of renal vascular dynamics (13, 14) this may be interpreted in the following manner. If the increase in the diodrast clearance and, therefore, in the renal blood flow, was accompanied by an increase in caliber of the afferent and efferent arterioles of

[4] In observations made recently in normal dogs with explanted kidneys the variations in diodrast clearance have been found to parallel those in renal blood flow.

the glomeruli, then the relatively greater increase in the inulin clearances suggests that the efferent arterioles underwent less dilatation than the afferent ones, with a consequent increase in intraglomerular pressure, and rise in the fraction of plasma water filtered.

DISCUSSION

In considering all of the observations which have been described thus far with respect to the blood flow and functional integrity of the kidneys of dogs with chronic experimental hypertension, whether of renal or neurogenic origin, it becomes apparent that no significant abnormalities have been detected in these animals, by the methods employed, to distinguish them from normal dogs. Corcoran and Page (15) have also noted the persistence of a normal renal blood flow in dogs with experimental renal hypertension. It may be concluded, therefore, that measurable and permanent reduction of renal blood flow is not the mechanism by which chronic experimental hypertension is maintained.

Although this conclusion may not necessarily be applicable to essential hypertension as it occurs in man, there are certain similarities between these two types of hypertension which are noteworthy. Normal "effective" renal blood flows have been found in patients who have had essential hypertension for relatively short periods of time (16, 17, 18). It has also been demonstrated (19) that the renal blood flow in hypertensive patients may be increased through the induction of pyrogenic reactions. However, the course of essential hypertension in man is usually characterized by progressive reduction in renal blood flow, which, when the comparative life spans of man and dog are considered, might have been expected in our dogs, if experimental and essential hypertension are identical in nature.

SUMMARY

1. Simultaneous determinations of the renal clearances of diodrast, inulin and urea were made in six dogs during periods of low and high protein intake. Two of the dogs had chronic hypertension of renal origin, two had chronic neurogenic hypertension, and two had normal blood pressures.

2. The clearance values of all the substances under consideration were much greater during the periods of high protein intake than during the

low ones. The inulin and urea clearances were increased to a relatively greater degree than were the diodrast clearances.

3. No qualitative differences were noted in the response of the normal, renal hypertensive and neurogenic hypertensive dogs to the changes in diet.

4. No significant anatomical abnormalities were found in the blood vessels, kidneys, or other organs of the dogs at post-mortem examination.

5. It is concluded that the blood flow and functional activities of the kidneys of dogs with chronic experimental hypertension may be unimpaired, and may react to the stimulus of increased dietary protein in a manner which is indistinguishable from the normal.

BIBLIOGRAPHY

1. Richards, A. M., Westfall, B. B., and Bott, P. A.: *Proc. Soc. Exp. Biol. & Med.*, **32:** 73, 1934.
2. Shannon, J. A.: *Am. J. Physiol.*, **112:** 405, 1935.
3. Shannon, J. A., and Smith, H. W.: *J. Clin. Invest.*, **14:** 393, 1935.
4. Elsom, K. A., Bott, P. A., and Shiels, E. H.: *Am. J. Physiol.*, **115:** 548, 1936.
5. Smith, H. W., Goldring, W., and Chasis, H.: *J. Clin. Invest.*, **17:** 263, 1938.
6. White, H. L., and Heinbecker, P.: (a) *Proc. Soc. Exp. Biol. and Med.*, **43:** 1, 1940. (b) White, H. L.: *Am. J. Physiol.*, **130:** 464, 1940.
7. Corcoran, A. C., Smith, H. W., and Page, I. H.: *Am. J. Physiol.*, **134:** 333, 1941.
8. Alving, A. S., Rubin, J., and Miller, B. F.: *J. Biol. Chem.*, **127:** 609, 1939.
9. Goldring, W., Chasis, H., Ranges, H. A., and Smith, H. W.: *J. Clin. Invest.*, **19:** 739, 1940.
10. Alpert, L. K.: *Bull. Johns Hopkins Hosp.*, **68:** 522, 1941.
11. Jolliffe, H., and Smith, H. W.: *Am. J. Physiol.*, **99:** 101, 1931.
12. Shannon, J. A., Jolliffe, N., and Smith, H. W.: *Am. J. Physiol.*, **101:** 625, 1932.
13. Smith, H. W., Chasis, H., Goldring, W., and Ranges, H. A.: *J. Clin. Invest.*, **19:** 751, 1940.
14. Lamport, H.: *J. Clin. Invest.*, **20:** 545, 1941.
15. Corcoran, A. C., and Page, I. H.: *Am. J. Physiol.*, **135:** 361, 1942.
16. Goldring, W., Chasis, H., Ranges, H. A., and Smith, H. W.: *J. Clin. Invest.*, **20:** 637, 1941.
17. Friedman, M., Selzer, A., and Rosenblum, H.: *J. A. M. A.*, **117:** 92, 1941.
18. Foa, P. P., Woods, W. W., Peet, M. W., and Foa, H. L.: *Arch. Int. Med.*, **69:** 822, 1942.
19. Chasis, H., Ranges, H. A., Goldring, W., and Smith, H. W.: *J. Clin. Invest.*, **17:** 683, 1938.

ENDOCARDIAL FIBROELASTOSIS (SO-CALLED FETAL ENDOCARDITIS)

A Report of Two Cases Occurring in Siblings

TOBIAS WEINBERG, M.D., and ALBERT J. HIMELFARB, M.D.

From the Laboratories and Medical Service of Sinai Hospital, Baltimore, Maryland

Received for publication March 25, 1943

The literature on the subject of so-called fetal endocarditis was ably reivewed recently by Gross (1) and, therefore, need not be repeated. It suffices to state that an analysis of the literature up to that time (1940) failed to reveal any unrefutable evidence for the support of the thesis of fetal endocarditis. It appears more likely that the endocardial changes seen in these cases can be explained better on the basis of a developmental defect.

The purpose of this presentation is (1) to report the occurrence of two cases of endocardial fibroelastosis (a much better term in view of the lack of inflammatory stigmata) involving the mural endocardium alone and not the valves, and occurring in siblings (a hitherto unrecorded phenomenon), and (2) to further substantiate the non-inflammatory character of the lesion.

REPORT OF CASES

Case 1. J. H., age 4½ months: The patient was the second child born to the parents, the first being alive and well. She was admitted to the Hospital because of continued weight loss and failure to eat, both of about one month duration. The prenatal and natal history were both negative and the delivery was entirely normal. She cried spontaneously and no cyanosis was noted at that time. However, the mother reported that the child had always seemed short of breath, this observation beginning shortly after birth. During the month before admission the child failed to feed properly and lost weight. The child's lips became cyanotic upon exertion or crying.

Physical Examination revealed a well developed infant manifesting no respiratory distress or cyanosis. A slight bulge was noted above and medial to the left nipple. The excursion of the right side of the chest appeared to be greater than that of the left. The percussion note over the left lung was impaired over the anterior chest close to the left cardiac border. Examination of the heart revealed it to be enlarged to the left. There were no murmurs or arrhythmia.

Laboratory Data: Red blood cells 4,300,000; Hemoglobin 12 grams; White blood cells 7,600; differential, polymorphonuclears 38 per cent and lymphocytes 58 per cent. Fluoroscopic examination as well as an x-ray of the chest showed a markedly enlarged heart, spherical in shape, which was interpreted as indicating a congenital lesion. The electrocardiogram showed a tendency to right axial deviation and was interpreted as showing myocardial involvement, probably associated with congenital heart disease.

Course in Hospital: During the first dew days the child had episodes of tachypnea. Five days after admission she became dyspneic and began to cough. The diaphragm showed great retraction with respiration. The precordial bulge seemed to increase and the child became cyanotic. The temperature rose to 103°F. and the respirations to 56 per minute. She was given sulfathiazole, but did not respond and died 6 days after admission to the Hospital.

Gross Necropsy Findings

The autopsy was performed by Dr. Irving Mauss.

The body was that of a well developed and fairly well nourished, approximately 5 months old, female infant in whom the lips and fingernail beds were moderately cyanotic. The description will be limited to the positive findings. The heart was markedly enlarged and weighed 90 grams. (The average normal weight for this age is 22 grams.) The foramen ovale was barely patent. The ductus Botalli was closed. The striking abnormality in the heart was limited to the left ventricle which was markedly dilated and moderately hypertrophied (Fig. 1). The endocardium of this chamber showed a diffuse grayish white thickening. The trabeculae carneae were partially flattened in the anterior and posterior walls. All valve cusps and leaflets as well as the valve rings were normal. The portion of the aorta proximal to the obliterated ductus arteriosus was distinctly narrowed and compared in size with the common carotid artery. The lungs were edematous.

Microscopic Findings

The thickening of the endocardium of the left ventricle was found to be due to a fibroelastic hyperplasia which was clearly demonstrable with the Weigert elastica-van Gieson stain (Fig. 2). The thin walled venous channels in the myocardium were greatly dilated and distended with blood. The findings in the other organs were non-contributory and only corroborated the gross findings.

Case 2. C. E. H., age 3½ months: This infant, the third born to the parents, was delivered one month prematurely. The pregnancy was otherwise completely uneventful. There were no difficulties associated with the delivery. The child was well until one month before admission. At that time the child did not feed well and breathed rapidly and with difficulty. These manifestations persisted from that time on, periodic vomiting occurring and increasing in frequency. Coughing paroxysms appeared and increased in frequency. It was also noted that the baby turned blue on exertion.

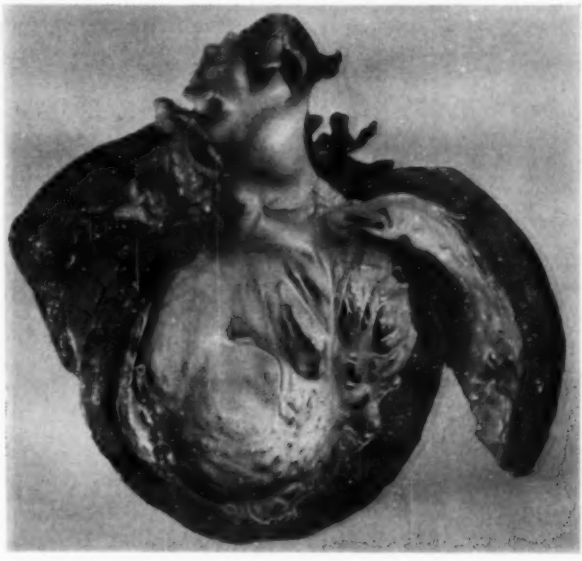

FIG. 1. Heart in Case I, showing the hypertrophy and dilatation of the left ventricle and the opaque thickening of the endocardium.

Physical Examination revealed a slightly cyanotic, dyspneic, male infant in a state of fair nutrition. The cyanosis was immediately increased by any exertional effort. There was marked retraction of the diaphragmatic attachments upon inspiration. The sternal region appeared to bulge. The heart was found to be enlarged, the left border being in the anterior axillary line and the right border extending 1.5 cm. to the right of the sternum. The heart was globular in outline to percussion. The rate was rapid and the rhythm regular. No murmurs were heard. The right lung was clear to percussion and auscultation. The left lung appeared to be compressed by the heart, dullness being obtained by percussion

and tubular breath sounds upon auscultation. The liver edge was felt 1 finger-breadth below the costal margin. There was no clubbing of the extremities.

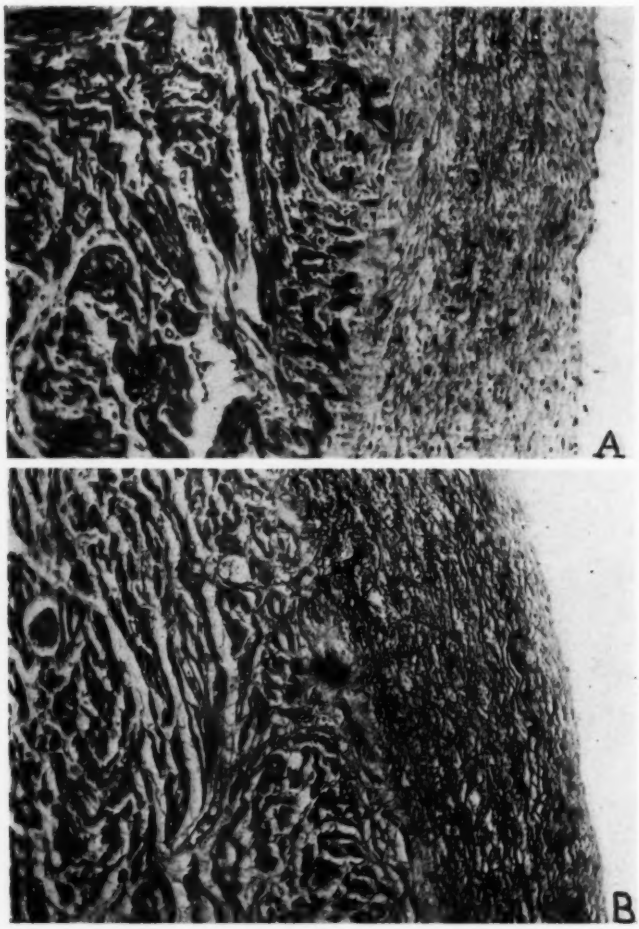

FIG. 2. (A) Shows the thickened endocardium (H. & E. stain). (B) Shows the elastic nature of the endocardial thickening. (Weigert's elastica-Van Gieson stain).

Laboratory Data: Hemoglobin 12.8 grams; Red blood cells 4,570,000; White blood cells 12,800; differential, polymorphonuclears 85 per cent; lymphocytes

12 per cent; and monocytes 3 per cent. X-ray of the chest showed a marked globular enlargement of the heart.

Course in Hospital: The patient's course was rapidly downhill. The tachypnea and dyspnea increased and the child was placed in an oxygen tent. He took feedings poorly. Alterations in breath sounds were heard over the upper portion of the right anterior and posterior chest walls and posteriorly over the left upper chest. The heart findings did not change. Digalen was given to no avail and the patient died three days after admission to the Hospital.

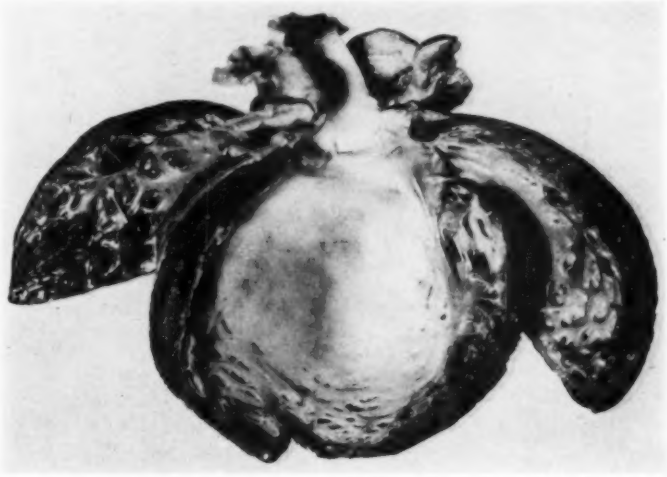

FIG. 3. Heart in Case II, showing the hypertrophy and dilatation of the left ventricle and the opaque thickening of the endocardium.

Gross Necropsy Findings

The body was that of a normally developed, 3½ months old, male infant whose lips and fingernail beds were deeply cyanotic. The contributory positive findings were in the heart and lungs. The heart was greatly enlarged, weighing 75 grams. (The normal weight for this age is about 20 grams.) The foramen ovale was closed. The right ventricle was moderately dilated and hypertrophied. The myocardium measured 4 mm. in thickness. Tiny foci of endocardial thickening were present in both the right auricle and ventricle. The left auricle contained broad, plaque-like areas of yellowish endocardial thickening. The left ventricle was enormously dilated and moderately hypertrophied, the myocardium measuring 7 mm. in thickness (Fig.

3). The endocardial surface of practically the entire left ventricle was grayish white and opaque. In some areas the endocardium measured as much as 1 mm. in thickness. The trabeculae carneae were less conspicuous than normally. The myocardium appeared normal. The ductus Botalli was closed. The aorta was of normal width, as was the aortic valve ring.

The lungs exuded a moderate amount of frothy fluid from their cut surfaces. A similar fluid was present in the trachea and bronchi.

Microscopic Findings

By means of the Weigert elastica-van Gieson stain the thickening of the endocardium of the left ventricle and auricle was demonstrated as being due to an increase mainly in elastic fibres with some increase in collagenous fibres. No alterations were found in the myocardium or mitral valve leaflet, although collections of inflammatory cells were particularly sought. Sections of the lungs revealed the presence in the alveoli of edema fluid and macrophages as well as scattered erythrocytes.

DISCUSSION

The similarities of the two cases are striking. Both were spontaneous, uneventful deliveries and consecutive off-spring of the same parents, neither of whom shows any physical abnormalities. The course of pregnancy was uneventful in both cases. Both children lived only a few months and during their brief period of survival pursued similar courses. The anatomical findings varied mainly in the degree and extent of involvement of the endocardium. In the first case, the endocardial thickening was limited to the left ventricle; in the second case the lesion was more extensive and involved mainly the left ventricle but also, focally, the other chambers of the heart. In connection with Case 1 there was another congenital defect, namely, coarctation of the aorta. The association of the two developmental defects has been noted in a case reported by Levine (2). In neither case was there any evident lesion of the myocardium. The defect was limited to an extensive fibroelastosis involving only the endocardium.

Böger (3) has expressed the opinion that endocardial fibroelastosis is produced by an overstretching of the endocardium in accompaniment with dilatation of the involved chamber. The absence of this

lesion in most cases of heart failure due to various causes, but particularly in cases of hypertension, seems to militate against this theory. A section of the thickened endocardium of the left ventricle taken from a case of healed infarction due to coronary artery occlusion revealed a similar fibroelastic thickening of the endocardium, but in this instance there was underlying fibrous replacement of the myocardium. This is unlike the findings in the two cases reported here and requires a different explanation for the thickening found in infants from that frequently found in cases of healed infarction.

Whatever the etiology of the endocardial fibroelastosis, its presence can be used to explain the failure of the ventricle. Wearn (4, 5) has beautifully demonstrated the intramyocardial circulation and its relationship to the chambers of the heart. With the development of such a thick fibroelastic layer as was present in the two cases persented here, one can well postulate some interference with the emptying of the arterio-luminal vessels into the ventricle because of constriction of their orifices by the elastic tissue. Thus, with the establishment of obstruction to the flow of blood, there would develop a dilatation of the intramyocardial capillaries, which would be accompanied by a partial stasis of the blood stream. The myocardium would therefore be subject to some degree of anoxemia, which would be reflected in a dilatation and ultimate failure of the ventricle due to failure of the muscle to receive sufficient oxygenation for normal metabolism, and would be expected to interfere with normal contraction. This could conceivably result in death before histologic myocardial changes would have time to take place. The dilatation of the myocardial vessels was evident in one of the cases reported, even though no particular pains were taken to prevent shrinkage by fixation in a special fixative. It is possible that had the infants lived long enough, myocardial alterations as observed by Gross (1) might have developed and so might have supported his thesis that the myocardial changes are secondary to "obliteration of arterioluminal, arteriosinusoidal and thebesian vessels by the marked parietal endocardial fibrosis."

SUMMARY

Two cases of fibroelastosis of the endocardium occurring in siblings are presented. This, together with the lack of history of any type of infection in the mother during both periods of pregnancy, militates

strongly against the concept of fetal endocarditis as implying an intrauterine infection and just as strongly supports the suspicion of an inherent developmental defect. Further support for the latter theory is seen in the lack of inflammatory stigmata in either the endo- or myocardium.

An explanation is offered for the ultimate left heart failure in these cases.

Since this paper was prepared for publication our attention has been called to a monograph by Dr. Douglas P. Murphy of the University of Pennsylvania, entitled "Congenital Malformations" (1940). According to his statistical analyses the same congenital defect tends to recur in siblings in more than 50 per cent of instances. This would tend to further substantiate our conclusion that fibroelastosis of the endocardium in infants is a developmental defect and non-inflammatory in nature.

REFERENCES

1. Gross, P.: Concept of Fetal Endocarditis. *Arch. Path.*, **31:** 163, 1941.
2. Levine, H. D.: Cardiac Hypertrophy in Infancy in Coarctation of Aorta. *Am. J. Dis. Child.*, **48:** 1072, 1934.
3. Böger, A.: Über die Endocardsklerosen. *Beitr. z. path.* Anat. u. z. allg. Path., **81:** 441, 1929.
4. Wearn, J. T.: Observations on the Morphology and Functions of some of the Components of the Coronary Circuit. *Bull. Johns Hopkins Hosp.*, **68:** 353, 1941.
5. Wearn, J. T.: Alterations in the Heart Accompanying Growth and Hypertrophy. *Bull. Johns Hopkins Hosp.*, **68:** 363, 1941.

BOOKS RECEIVED FOR REVIEW

Principles and Practice of Obstetrics. By JOSEPH B. DELEE and J. P. GREENHILL. 8th ed. 1101 pp. Illus. $10.00. W. B. Saunders Company, Philadelphia and London, 1943.

Clinical Diagnosis by Laboratory Methods. By JAMES CAMPBELL TODD and ARTHUR HAWLEY SANFORD. 10th ed. 911 pp. Illus. $6.00. W. B. Saunders Company, Philadelphia and London, 1943.

Renal Lithiasis. By CHARLES C. HIGGINS. 140 pp. Illus. $3.00. Charles C. Thomas, Springfield, Illinois, 1943.

Burns, Shock, Wound Healing and Vascular Injuries. Prepared under the Auspices of the Committee on Surgery of the Division of Medical Sciences of the National Research Council. 272 pp. Illus. $2.50. W. B. Saunders Company, Philadelphia and London, 1943. (Military Surgical Manuals, Vol. 5.)

Chemotherapy of Gonococcic Infections. By RUSSELL D. HERROLD. 137 pp. $3.00. The C. V. Mosby Company, St. Louis, 1943.

Introduction to Organic and Biological Chemistry. By L. EARLE ARNOW and HENRY C. REITZ. 736 pp. Illus. $4.25. The C. V. Mosby Company, St. Louis, 1943.

Principles and Practice of War Surgery. By J. TRUETA. 441 pp. Illus. $6.50. The C. V. Mosby Company, St. Louis, 1943.

Gynecology, with a section on Female Urology. By LAWRENCE R. WHARTON. 1006 pp. Illus. $10.00. W. B. Saunders Company, Philadelphia and London, 1943.

Operating Room Technique. By EDYTHE LOUISE ALEXANDER. 392 pp. Illus. $3.75. The C. V. Mosby Company, St. Louis, 1943.

Synopsis of Diseases of the Skin. By RICHARD L. SUTTON and RICHARD L. SUTTON, JR. 481 pp. Illus. $5.50. The C. V. Mosby Company, St. Louis, 1942.

Laboratory Experiments in Physiology. By W. D. ZOETHOUT. 3rd ed. 256 pp. Illus. $2.25. The C. V. Mosby Company, St. Louis, 1943.

Behind the Sulfa Drugs. By IAGO GALDSTON. 174 pp. $2.00. D. Appleton-Century Company, New York and London, 1943.

Doctor in the Making. By ARTHUR W. HAM and M. D. SALTER. 179 pp. Illus. $2.00. J. B. Lippincott Company, Philadelphia, 1943.

Standard Nursing Procedures of the Department of Hospitals, City of New York. Prepared by the Committee on Nursing Standards, Division of Nursing, Department of Hospitals. Mary Ellen Manley, Director. 436 pp. Illus. $3.25. The Macmillan Company, New York, 1943.

Neurosurgery and Thoracic Surgery. Prepared and Edited by the Subcommittees on Neurosurgery and Thoracic Surgery of the Committee on Surgery of the Division of Medical Sciences of the National Research Council. 310 pp. Illus. $2.50. W. B. Saunders Company, Philadelphia & London, 1943. (Military Surgical Manuals, Vol. 6.)